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THE VITAMIN CONTENTS OF PHILIPPINE FOODS, IV

VITAMINS A AND B_1 IN VARIOUS FRUITS AND VEGETABLES

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This paper is a continuation of our work on the vitamin contents of Philippine vegetables, fruits, and other products. Three papers [1] have already appeared and this makes our fourth report.

Previous work on the vitamin contents of some Philippine edible products has also been carried out by various other investigators.[2]

In the Philippines the diet of the masses consists largely of polished rice, which is deficient in vitamin B_1. As vegetables and fruits, more or less, are also included in the diet, a knowledge of the vitamin contents of these products is of considerable importance, for such information is helpful in correcting deficiencies in the diet.

MATERIALS

The materials used in this investigation consisted of various Philippine fruits and vegetables. Most of them were purchased in the public markets of Manila, though some were obtained from

[1] Hermano, A. J., Philip. Journ. Sci. 41 (1930) 387. Hermano, A. J., and G. Sepulveda, jr., Philip. Journ. Sci. 53 (1934) 379; 54 (1934) 61.

[2] Jesus, Francisco de, Philip. Agr. 5 (1917) 533. Santos, F. O., Am. Journ. Physiol. 59 (1922) 310. Acuña, E. M., Philip. Agr. 12 (1923) 293. Santos, F. O., and S. Santos, Philip. Journ. Sci. 30 (1926) 307. Santos, F. O., and E. G. Collado, Philip. Agr. 16 (1928) 513. Sherman, Hartley Embrey, Philip. Journ. Sci. 38 (1929) 1, 9. Hermano, A. J., Philip. Journ. Sci. 41 (1930) 387. Lara, C. B., and Manuel Nicolas, Philip. Is. Med. Assoc. Journ. 10 (1930) 263.

the provinces. Fourteen samples were tested for the vitamin A content and ten were examined for vitamin B_1. The products tested were as follows:

ACHRAS ZAPOTA Linn.; local names, chico and siku.

The chico is one of the most palatable of Philippine fruits. It is somewhat spherical and oblong in shape and rather brown in color. The soft juicy flesh has a sweet and characteristic odor and flavor, although in some fruits the flesh is rather gritty or sandy.

ARTOCARPUS CHAMPEDEN (Lour.) Spreng.; local name, lemosa.

The lemosa is a fruit that has a flavor somewhat similar to that of jack fruit. It contains many seeds. The edible flesh has a yellow color and an exceedingly sweet aromatic taste and odor.

BRASSICA CHINENSIS Linn.; local name, pechai.

Pechai is a vegetable that is grown rather extensively. The succulent leaves and tops are eaten like spinach. Locally it is cooked alone or boiled with other vegetables and meat.

CERATOPTERIS THALLICTROIDES (Linn.) Brongn.

This is an edible fern the leaves and tops of which may be used as a leafy vegetable, as "greens," or for salads.

CORCHORUS OLITORIUS Linn.; local names, saluyot, tagabang, lamhay, and pasau-na haba.

Saluyot is an annual herb with pointed, toothed leaves, and small yellow flowers. It is extensively used in the northern part of the Philippines. The tender leaves and tops are boiled and eaten as vegetables.

CUCURBITA MAXIMA Duchesne; local names, calabaza, and kalabasa-napula.

Squash is an herbaceous annual plant that is tendril-climbing. The large fruits, flowers, tender leaves, and tops are edible. The tender leaves and tops are boiled and eaten as vegetables.

IPOMOEA BATATAS (Linn.) Poir.; local name, kamote.

Kamote is a perennial, creeping herbaceous vine. A resinous latex flows when the vine and stem are cut. The fleshy roots, which are edible, occur in various shapes, sizes, and colors. They contain about 20 per cent starch. The leaves and tops are boiled and eaten as vegetables or as salad.

IPOMOEA REPTANS (Linn.) Poir.; local names, kangkong and tangkong.

Kangkong is a trailing vine that grows in swampy land and in shallow, stagnant water. It is usually not cultivated but grows wild. It is frequently sold in the local markets. The tender leaves and tops are boiled and eaten as vegetables or as salad.

MANGIFERA INDICA Linn.; local name, mangang-kalabau.

The mango grows on a tree that is medium to rather large in size. The ripe fruits are yellow and of different sizes. The fruit has a large seed that is covered with a sweet, juicy pulp. The variety known as "mangang-kalabau" was used in these experiments.

MUSA SAPIENTUM Linn. var. LACATAN (Blanco) Teodoro; local name, saguing na lakatan.

The lakatan is one of the best varieties of bananas grown in the Philippines.

PSOPHOCARPUS TETRAGONOLOBUS (Linn.) DC.; local names, cigarillas, segadilla, and kalamismis.

The segadilla is a perennial vine twining usually on a fence or bamboo used for support. The tender winged pods are boiled and eaten as vegetables or they may be eaten as salad.

SANDORICUM KOETJAPE (Burm. f.) Merr.; local names, santol and santor.

The santol is a medium-sized tree. The fruit is rather round but somewhat flat at the top and bottom. It is light brown in color and contains a juicy pulp. There are both sweet and sour varieties with characteristic flavor. It is an excellent fruit for making jelly.

ZEA MAYS Linn.; local names, maize and kamais.

The local varieties of corn are mostly white or yellow. In some provinces the people use corn in addition to rice as the staple diet.

ANONA MURICATA Linn.; local names, guanabano and guayabano.

Guayabano is a small tree that has rather large oblong fruits. These fruits have a green color and soft spines. The ripe fruit has white juicy flesh that is fibrous and has a characteristic flavor.

BRASSICA INTEGRIFOLIA (West.) O. E. Schulz; local name, mostaza.

Mustard is an herbaceous annual plant. It is cultivated for its leaves which are boiled and eaten as a green vegetable.

SOLANUM MELONGENA Linn.; local name, talong.

The eggplant is an herbaceous annual plant that is cultivated extensively in the Philippines. The fruits vary in shape from rather round to oblong or cylindrical. It is one of the commonest vegetables sold in the local markets.

EXPERIMENTAL PROCEDURE

As in our previous experiments we used the general methods for the biological anlysis of foods. The basal ration in the vitamin A test was modified in that a standard brand of irradiated yeast was used instead of the tikitiki extract formerly employed. Most of the samples were dried in an electric oven controlled at 80° C. They were then powdered and mixed uniformly with the basal ration.

A group of albino rats, weighing about 40 grams each, were given ad libitum a basal ration deficient in vitamin A, and another group was given a basal ration deficient in vitamin B_1. When the animals showed definite symptoms of xerophthalmia (vitamin A deficiency) or beriberi (vitamin B_1 deficiency) the deficient basal rations were supplemented with portions of the samples to be tested. Powdered samples were added in sufficient amount to make the altered basal ration contain 10 per cent of the powdered sample, though in one case the basal ration contained 15 per cent.

Some test animals were given daily a definite quantity (2 grams) of fresh fruit as a supplement to the deficiency in the basal ration. In experiments with guanabano 2 cubic centimeters of the strained pulp and juice were fed daily to each rat.

The time required to effect a cure for vitamin deficiency was usually about 28 days. During this period the symptoms for vitamin deficiency gradually disappeared and finally the rats appeared to be normal again.

The results of our experiments are recorded in Tables 1 and 2.

SUMMARY

A number of Philippine fruits and vegetables were tested biologically for their vitamin (A and B_1) contents. The results showed that these products vary in their biological value as sources of vitamins.

In testing for vitamin A the rats fed with kangkong, as a supplement to the basal ration, gave the greatest average daily gain in weight (4.03 grams).

Other samples that gave satisfactory results and appeared to be good sources of vitamin A were lemosa fruit, the leaves of pechai, fern, saluyot, squash, and sweet potato, pods of segadilla, and corn. Rats fed with these products gained 2 to 3 grams daily.

When fed with frozen mango the rats had an average daily gain in weight of 1.88 grams. This would indicate that vitamin A in mangos is not greatly affected by freezing.

In general, those materials that had a green or yellow color appeared to be good sources of vitamin A.

Of the samples that were tested biologically for vitamin B_1 the tender leaves and tops of the squash gave the best results. The rats gained an average of 3.59 grams daily in weight.

Rats fed with the leaves and tops of mustard, saluyot, and sweet potato, and the fruit of santol gained 2.05 to 2.75 grams daily. These products were, therefore, found to be good sources of vitamin B_1.

Only three products were found to be good sources of both vitamins A and B_1, and these were the tender leaves and tops of saluyot, squash, and sweet potato.

TABLE 1.—*Results of feeding various fruits and vegetables to albino rats for vitamin A content.*[a]

Names of fruits and vegetables.		Parts used.	Amount given.	Average daily gain in weight of rats.	Remarks.
Scientific.	Common.				
Achras zapota Linn	Chico, siku	Ripe fruit dried at 80° C.	The basal ration contained 10 per cent of the sample.	g. 1.55	Xerophthalmia disappeared and the rats gained in weight gradually and became normal.
Artocarpus champeden (Lour.) Spreng.	Lemosa	Pulp of the ripe fruit (fresh).	2 grams daily.	2.96	Xerophthalmia was cured. The rats gained in weight and became active.
Brassica chinensis Linn	Pechai, selgas, messay.	Tender leaves and tops dried at 80° C.	The basal ration contained 10 per cent of the sample.	2.00	The increase in weight and the disappearance of xerophthalmia were gradual. The rats became normal.
Ceratopteris thallictroides (Linn.) Brongn.	Fern	do	do	2.35	Xerophthalmia was cured gradually. The rats gained in weight and became normal.
Corchorus olitorius Linn	Saluyot, tagabang, pasau-na-haba.	do	do	2.18	The rats gained in weight and became normal. Xerophthalmia disappeared.
Cucurbita maxima Duchesne	Squash, calabaza, kalabasa.	do	do	2.64	The rats gained in weight gradually and xerophthalmia disappeared. The animals became normal.
Ipomoea batatas (Linn.) Poir	Kamote	do	do	2.09	The rats gained in weight gradually and were cured of xerophthalmia. The animals became normal.
Do	Kamote, sweet potato.	Fleshy roots with white contents, dried at 80° C.	do	(b)	The amounts might not have been sufficient to cure xerophthalmia.
Ipomoea reptans (Linn.) Poir	Kangkong, tangkong.	Tender leaves and tops dried at 80° C.	do	4.03	Rapid increase in weight and disappearance of xerophthalmia were noted. The rats became active and normal.
Mangifera indica Linn	Mangang-kalabau, mango, páho.	Frozen pulp of ripe fruit	2 grams daily	1.88	The rats gained in weight gradually and xerophthalmia disappeared. The rats became active and normal.

Musa sapientum Linn. var. *lacatan* (Blanco) Teodoro.	Banana, lakatan	Fresh ripe fruit	do	1.74	Xerophthalmia disappeared gradually and the animals gained in weight. The rats became normal.
Psophocarpus tetragonolobus (Linn.) DC.	Cigarillas, segadilla, kalamismis.	Tender winged pods dried at 80° C.	The basal ration contained 10 per cent of the sample.	2.38	The rats gained in weight gradually and xerophthalmia disappeared. The animals became normal.
Sandoricum koetjape (Burm. f.) Merr.	Santol, santor	Pulp of ripe fruits dried at 80° C.	do	(•)	The three rats showed slow disappearance of xerophthalmia.
Zea mays Linn	Maize, corn, kamais	Powder of whole dried and matured grain.	do	2.77	The rats gained in weight gradually and xerophthalmia disappeared. The animals became normal.

[a] Each sample was tested with three white rats.
[b] Three rats failed to gain weight.
[c] One rat gained in weight by 0.73 g. The two others failed to gain in weight.

TABLE 2.—*Results of feeding various fruits and vegetables to albino rats for vitamin B_1 content.*[a]

Names of fruits and vegetables.		Parts of plant used.	Amount fed.	Average daily gain in weight of rats.	Remarks.
Scientific.	Common.				
Anona muricata Linn.	Guanabano, guayabano.	Pulp and juice of the ripe fruit (fresh).	2 cc of the strained pulp and juice daily.	g. 1.94	The rats gained in weight gradually, and the symptoms disappeared. The rats became active and normal.
Brassica integrifolia (West.) O. E. Schulz.	Mustard, mostaza	Tender leaves and tops dried at 80° C.	The basal ration contained 10 per cent of the sample.	2.18	The symptoms disappeared and the rats gained in weight rapidly. The animals became active and normal.
Corchorus olitorius Linn.	Saluyot, tagabang, pasau-na-haba.	do	do	2.58	The rats recovered rapidly and gained in weight continually. The animals became active and normal.
Cucurbita maxima Duchesne	Squash, calabaza	do	do	3.59	The rats recovered from the disease rapidly and gained in weight continually. The animals became active and normal.
Ipomoea batatas (Linn.) Poir	Kamote, sweet potato, tigsi.	Fleshy root with white contents dried at 80° C.	do	(b)	The animals continued to show advanced vitamin B_1 deficiency.
Do.	do	Tender leaves and tops dried at 80° C.	do	2.75	Immediate disappearance of the symptoms of vitamin B_1 deficiency. The rats gained in weight rapidly and became normal.
Musa sapientum Linn. var. *lacatan* (Blanco) Teodoro.	Lakatan, bañgaran	Fresh ripe fruit	2 g of the fruit daily	1.74	The symptoms of the vitamin B_1 deficiency disappeared gradually. The animals gained in weight and became normal.
Sandoricum coetjape (Burm.f.) Merr.	Santol, santor	Meat of ripe fruit, dried at 80° C.	The basal ration contained 10 per cent of the sample.	2.05	The animals recovered from vitamin B_1 deficiency and became normal.

Solanum melongena Linn	Eggplant,[a] talong, tarong.	Immature tender fruit dried at 80° C.	The basal ration contained 15 per cent of the sample.	1.46	Gradual disappearance of vitamin B_1 deficiency was noted. The animals gained in weight and became normal.
Zea mays Linn	Corn (yellow), maize, kamais.	Whole matured grains, powdered.	The basal ration contained 10 per cent of the sample.	1.65	The rats gained in weight gradually and the symptoms of vitamin B_1 deficiency disappeared. The animals became normal.

[a] Each sample was tested with three rats except the fleshy root of sweet potato for which six rats were used.
[b] The rats were losing weight.
[c] Three rats died with basal ration containing 10 per cent of the sample.

TREMATODE PARASITES OF PHILIPPINE VERTEBRATES, VII

ADDITIONAL RECORDS OF NEW SPECIES

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THREE PLATES

Family OPISTHORCHIIDÆ Braun, 1901

OPISTHORCHIS OPHIDIARUM sp. nov. Plate 1, fig. 1.

A single specimen of this trematode was found in the intestine of a water snake, *Lapemis hardwickii* Gray. The liver of the snake was not examined; if the intestine should prove to be the normal habitat of the parasite, then it is one of the very few exceptions among the members of the genus *Opisthorchis* that occur outside of the bile ducts.

This fluke is the second species of the genus *Opisthorchis* to be reported from the Philippines, the first one being *O. wardi* Wharton, 1921. In the shape of its body it bears a superficial resemblance to *O. anatinus* Yamaguti, 1933, and to *Notaulus asiaticus* Skrjabin, 1913, which, according to Morgan (1927), should be included in the genus *Opisthorchis*. In the possession of cuticular spines it is similar to *O. viverrini* (Poirier, 1886); *O. piscicola* (Odhner, 1902); *O. noverca* Braun, 1903; and *O. pedicellata* Verma, 1927. In the shape of its testes it resembles *O. pedicellata* and *O. anatinus*. It differs, however, from these various species in having a long prepharynx. In the latter respect as well as in the presence of cuticular spines and in the shape and location of its ovary, testes, and excretory bladder, it is very closely related to *Gomtia piscicola* which, according to Thapar (1930), occupies an intermediate position between *Opisthorchis* and *Notaulus*. It may be differentiated from *Gomtia piscicola* by its more elongate shape, the more anterior location of its acetabulum, and the anterior extent of its vitelline glands, which reach to the anterior third of the body length.

Description.—Body very elongate, 8.5 millimeters in length by 0.40 millimeter in maximum diameter across ovary. Cuticle

armed with minute spines from anterior end to slightly beyond posterior third of body length. Oral sucker moderately developed, subterminal, 0.20 by 0.18 millimeter in size; acetabulum weak, in anterior ninth of body length, 0.10 by 0.11 millimeter in size. Oral sucker separated from pharynx by a prepharynx 0.23 millimeter long; pharynx 0.16 by 0.13 millimeter in size; œsophagus 0.10 millimeter long, bifurcates immediately in front of acetabulum; intestinal cæca reach to near posterior end of body.

Testes oval, postovarial, one immediately behind the other and almost touching, in posterior sixth of body length and confined between intestinal cæca; anterior testis 0.29 by 0.22, posterior testis 0.33 by 0.22 millimeter in size. Seminal vesicle prominently enlarged in diameter and much convoluted; ejaculatory duct unarmed. Common genital opening median, immediately preacetabular.

Ovary globular, to one side of median line, immediately pretesticular, 0.16 millimeter in diameter. Oviduct arises from mediolateral border of ovary; receptaculum seminis small, flask-shaped; Laurer's canal present. Shell gland diffuse, almost median, between ovary and anterior testis. Uterus very long, in transverse coils and mostly confined in space between ovary, intestinal cæca, and acetabulum. Vitelline glands finely granular, not distinctly divided into acini and lying along margins of body from immediately in front of level of ovary to slightly beyond junction of anterior and middle thirds of body length. Eggs typical in appearance; that is, they are operculated and possess a shouldered thickening into which the operculum fits; they measure 30 to 34 by 17 to 19 microns.

Excretory pore posteroterminal; excretory bladder straight, narrow in diameter, dorsal to testes and divides into two branches behind ovary.

Specific diagnosis.—Opisthorchis: Body very elongate, 8.5 by 0.40 millimeters in size; cuticle armed. Prepharynx present, 0.23 millimeter long. Acetabulum in anterior ninth of body length. Ovary and testes with smooth borders. Vitelline glands extend from immediately in front of level of ovary to slightly beyond junction of anterior and middle thirds of body length. Excretory bladder a narrow straight tube passing dorsal to testes. Ova 30 to 34 by 17 to 19 microns in size.

Host.—Lapemis hardwickii Gray.

Location.—Intestine.

Locality.—Paombong, Bulacan, Luzon.

Type specimen.—Philippine Bureau of Science parasitological collection No. 408.

Family ECHINOSTOMATIDÆ Looss, 1902

ECHINOSTOMA CHARADRII sp. nov. Plate 1, figs. 2 and 3.

This trematode is closely related to *Echinostoma batanguensis* Tubangui, 1932, but differs from it in having sixty-nine collar spines instead of seventy-two.

Description.—Body elongate, 6.8 to 8.8 millimeters in length by 2.1 to 2.5 millimeters in maximum diameter across middle of body length. Ventral surface from anterior end to anterior level of acetabulum armed with small spines. Oral sucker small, subterminal, 0.28 to 0.36 by 0.30 to 0.40 millimeter in size; acetabulum large, cup-shaped, 1.0 to 1.2 millimeters from anterior end, 1.3 to 1.6 by 1.0 to 1.6 millimeters in size. Head collar reniform, 1.07 to 1.35 millimeters across, with sixty-nine spines arranged in two alternating rows uninterrupted dorsally. The different groups of collar spines vary in size, the corner spines in some specimens being the larger, and in others some of the dorsal spines; the larger spines measure 125 by 16.5, the smaller ones 66.5 by 25 microns. Mouth ventroterminal; prepharynx absent; pharynx 0.27 to 0.33 by 0.20 to 0.25 millimeter in size; œsophagus 0.20 to 0.43 millimeter long, bifurcates immediately in front of acetabulum; intestinal cæca narrow in diameter, extend to near posterior end of body.

Testes tandem, small, with irregular outline but not lobed, postovarial; anterior testis measures 0.56 to 0.80 by 0.44 to 0.78, posterior testis 0.47 to 0.80 by 0.36 to 0.70 millimeter. Cirrus sac oval, 0.35 to 0.38 by 0.26 to 0.58 millimeter in size, dorsal to acetabulum, barely protruding anteriorly beyond anterior border of latter; incloses seminal vesicle, pars prostatica, and cirrus. Common genital opening median, behind œsophageal bifurcation, covered by acetabulum in ventral view.

Ovary transversely ovoid, median, equatorial or immediately preëquatorial, 0.32 to 0.36 by 0.46 to 0.63 millimeter in size. Shell gland prominent, slightly larger than ovary, between latter and anterior testis. Receptaculum seminis absent, Laurer's canal present. Uterus moderately developed, intercæcal, from ovary to posterior border of acetabulum. Vitellaria follicular, lying on lateral margins of body, from level of posterior border of acetabulum to posterior end of body; follicles of two sides

do not intermingle behind second testis. Eggs oval, operculated, thin-shelled, yellowish, 87 to 100 by 54 to 64 microns in size.

Excretory pore posterodorsal; excretory bladder with a terminal secondary bladder, divides into two branches behind second testis.

Specific diagnosis.—Echinostoma: Length 6.8 to 8.8, maximum diameter 2.1 to 2.5 millimeters. Cephalic collar 1.07 to 1.35 millimeters in diameter, armed with sixty-nine spines. Testes irregular in outline but not lobed. Eggs 87 to 100 by 54 to 64 microns in size.

Host.—Pluvialis fulvus.

Location.—Intestine.

Locality.—Baao, Camarines Sur, Luzon.

Type specimens.—Philippine Bureau of Science parasitological collection No. 324.

PEGOSOMUM BUBULCUM sp. nov. Plate 2, fig. 1.

The genus *Pegosomum* von Ratz, 1903, includes three species of flukes; namely, *P. saginatum* (von Ratz, 1898), *P. spiniferum* von Ratz, 1903, and *P. asperum* (Wright, 1879). The original description of *P. asperum* is not available to the writers so that the parasite in question can only be compared with the first two mentioned species. It differs from these in its smaller dimensions and in the posterior extent of its vitelline glands, which do not pass beyond the level of the second testis. In *P. saginatum* and *P. spiniferum*, according to von Ratz (1900) and Odhner (1910), respectively, the vitellaria extend to the posterior end of the body.

Description.—Body elongate, tapering towards both extremities more so anteriorly than posteriorly, 5.0 to 6.6 millimeters in length by 1.7 to 2.3 millimeters in maximum breadth across acetabulum or immediately in front of that organ. Entire body surface covered with spines, of which those anterior to the acetabulum are smaller and measure 10.5 to 20.8 by 4.0 to 11.2 microns; those posterior to the acetabulum reach a maximum size of 41.6 by 16.6 microns. Oral sucker weak, 0.14 to 0.15 millimeter across; acetabulum well developed, immediately preequatorial, 0.68 to 0.83 millimeter in diameter. Cephalic collar poorly developed, 0.34 to 0.36 millimeter across, armed with twenty-seven spines arranged in a single row uninterrupted dorsally. Corner collar spines, of which there are four on each side, larger and more or less bunched together, measuring 80 to 83 by 19 microns; lateral collar spines measure 71 to 77 by

19 to 23 and the dorsal spines 50 to 58 by 17 to 19 microns. Pharynx well developed, 0.26 to 0.28 by 0.15 to 0.17 millimeter in size; œsophagus 1.24 to 1.46 millimeters long, bifurcates immediately in front of acetabulum; intestinal cæca simple, slightly wavy in outline, moderately large in diameter, reaching to near posterior end of body.

Testes tandem, postovarial, postequatorial, dissimilar; anterior testis transversely elongate, boat-shaped, 0.38 to 0.41 by 1.18 to 1.52 millimeters in size; posterior testis rounded to ovoid, 0.57 to 0.82 by 0.57 to 1.20 millimeters in size. Cirrus pouch immediately preacetabular, submedian, 0.32 to 0.47 by 0.50 to 0.66 millimeter in size; incloses seminal vesicle, pars prostatica, and cirrus. Common genital pore median, in front of acetabulum, about midway between latter and œsophageal bifurcation.

Ovary small, equatorial, to right side of median line, 0.19 to 0.42 by 0.28 to 0.30 millimeter in size. Shell gland large, median, immediately in front of anterior testis. Uterus short to moderately long, mostly between cæca, anterior testis, and acetabulum. Vitellaria in small granular follicles, from posterior level of pharynx to anterior level of second testis; anteriorly the vitelline glands of both sides meet in the median line, but behind level of œsophageal bifurcation they separate and only occupy lateral margins of body. Transverse vitelline ducts immediately in front of first testis. Ova thin-shelled, yellowish to yellowish brown, operculated, 115.2 to 122.8 by 73 to 76.5 microns in size.

Excretory pore posteroterminal; excretory bladder divides into two branches behind second testis.

Specific diagnosis.—Pegosomum: Length 5.0 to 6.6, maximum breadth 1.7 to 2.3 millimeters; entire body surface covered with spines. Vitellaria from posterior level of pharynx to anterior level of second testis. Ova 115.2 to 122.8 by 73 to 76.5 microns in size.

Host.—Bubulcus coromandus.

Location.—Liver.

Locality.—Baao, Camarines Sur, Luzon.

Type specimens.—Philippine Bureau of Science parasitological collection No. 326.

Family DICROCŒLIIDÆ Odhner, 1911

PARADISTOMUM EXCALOTES sp. nov. Plate 2, fig. 2.

This is the third species of the genus *Paradistomum* to be described from the Philippines, the others being *P. gregarinum*

(Tubangui, 1928) Tubangui, 1929, and *P. paloensis* Tubangui, 1933. It differs from these two forms in being smaller and in the posterior extent of its cirrus pouch, which reaches the anterior border of the acetabulum. It is more closely related to *P. mutabile* (Molin, 1859), from which it can be differentiated, however, by the shape of its cirrus sac, which is cylindrical; by the position of its genital pore, which is opposite or immediately in front of the œsophageal bifurcation; and by the larger number of its vitelline follicles. In *P. mutabile,* according to Dollfus (1922), the cirrus sac is fusiform, the genital opening is ventral to the pharynx, and the vitellaria are composed on each side of only 10 to 12 large follicles.

Description.—Body ovoid, broader posteriorly than anteriorly, 1.1 to 1.8 millimeters in length by 0.44 to 0.80 millimeter in maximum diameter across posterior third of body length. Cuticle smooth. Oral sucker subterminal, 0.15 to 0.20 by 0.14 to 0.21 millimeter in size; acetabulum weak, between anterior and middle thirds of body length, 0.17 to 0.20 millimeter across. Pharynx partly overlapped by oral sucker, 0.070 to 0.075 by 0.055 to 0.060 millimeter in size; œsophagus 0.07 to 0.11 millimeter long; intestinal cæca characteristically wide in diameter, extending to from 0.06 to 0.10 millimeter from posterior end of body.

Testes generally smooth but sometimes with slightly indented margins, oval to subglobular, symmetrical, antovarial, immediately postacetabular, 0.11 to 0.17 by 0.09 to 0.12 millimeter in size. Cirrus sac cylindrical, 0.19 to 0.23 by 0.055 to 0.065 millimeter in size, reaching posteriorly to anterior border of acetabulum; incloses poorly developed seminal vesicle, pars prostatica, and cirrus. Common genital opening median, ventral to œsophageal bifurcation.

Ovary transversely oval, generally smooth but sometimes with slightly indented margins like testes, to one side of median line, immediately behind testes, generally smaller than latter but sometimes almost as large; measures 0.10 to 0.11 by 0.14 to 0.16 millimeter. Shell gland smaller than or as large as ovary, median or submedian; receptaculum seminis and Laurer's canal present. Uterus well developed, occupying most of space not otherwise occupied by other organs behind testes, thus crowding out vitelline glands and obscuring in ventral view the greater lengths of intestinal cæca. Vitellaria consist of moderately numerous follicles and occupy lateral margins of body external to cæca from anterior level of testes to junction between third and

last fourth of body length. Transverse vitelline ducts leave vitelline glands at level of ovary. Eggs oval, operculated, thick-shelled, yellowish to dark brown in color, 32.5 to 38.5 by 21 to 23 microns in size.

Excretory pore median, posterodorsal; excretory bladder voluminous, divides into two small branches behind shell gland.

Specific diagnosis.—Paradistomum: Length 1.1 to 1.8 millimeters, maximum diameter 0.44 to 0.80 millimeter. Cirrus sac cylindrical, 0.19 to 0.23 by 0.055 to 0.065 millimeter in size; reaches posteriorly to anterior border of acetabulum. Genital pore ventral to œsophageal bifurcation. Vitellaria consist of moderately numerous follicles. Eggs 32.5 to 38.5 by 21 to 23 microns in size.

Host.—Calotes calotes marmoratus.

Location.—Gall bladder.

Locality.—San Juan, Rizal, Luzon.

Type specimens.—Philippine Bureau of Science parasitological collection No. 412.

Family CLINOSTOMIDÆ Luehe, 1901

EUCLINOSTOMUM MULTICÆCUM sp. nov. Plate 3, fig. 2.

This parasite is the second metacercarial fluke to be reported from the fresh-water fish *Ophiocephalus striatus*, the first one being *Clinostomum dalagi* Tubangui, 1933. It belongs to the genus *Euclinostomum*, the members of which were separated by Travassos (1928) from the old genus *Clinostomum* Leidy, 1856, due to the presence of long cæcal diverticula. It differs from *Euclinostomum heterostomum* (Rudolphi, 1809) in the extent of its uterus which reaches anteriorly only slightly beyond the middle of the body length and not to the posterior border of the acetabulum as in the case of *E. heterostomum*. In that respect it resembles *E. clarias* (Dubois, 1929), a metacercaria found in the body cavity of another fish, *Clarias angolense*. Dubois' original description is not available to the writers, but according to Baer (1933) the genital organs of *E. clarias* are located in the posterior third of the body length, while those of the Philippine species are situated in the middle of the body length.

Description.—Body elongate, rounded at both ends, 10.5 to 13.0 millimeters in length by 3.7 to 4.5 millimeters in maximum breadth across middle of body length. Cuticle unarmed. Oral sucker terminal, weak, 0.5 to 0.7 millimeter across. Acetabulum well developed, in middle of anterior third of body length, meas-

ures 1.2 to 1.5 by 1.5 millimeters. A very small rudimentary pharynx is present; œsophagus a narrow canal, 0.12 to 0.25 millimeter long. Intestinal cæca prominent, reaching to near posterior end of body, with long external lateral diverticula in postacetabular region; some of the diverticula are forked distally and also reach to near posterior end of body.

Testes tandem, in posterior portion of middle third of body length; anterior testis almost U-shaped, 0.12 to 0.18 by 1.16 to 1.40 millimeters in size; posterior testis crescent-shaped, 0.24 to 0.27 by 0.64 to 0.95 millimeter.

Ovary very small, intertesticular, on right side of median line, 0.15 to 0.16 by 0.17 to 0.20 millimeter in size. Shell gland median, larger than ovary and also between testes. Uterine sac a median longitudinal tube, immediately in front of first testis and extending anteriorly to slightly beyond middle of body length; 1.5 to 1.8 millimeters long. Vitellaria profuse, occupy most of space in postacetabular region not otherwise occupied by other organs.

Excretory pore median, posterodorsal; a pair of prominent lateral excretory vessels lead to excretory pore.

Specific diagnosis.—Euclinostomum: Length 10.5 to 13.0, maximum diameter 3.7 to 4.5 millimeters. Intestinal cæca with long diverticula in postacetabular region. Genital organs in middle third of body length; anterior testis U-shaped, posterior testis crescent-shaped.

Host.—Ophiocephalus striatus.

Location.—Encysted in muscular tissues.

Locality.—Baao, Camarines Sur, Luzon.

Type specimens.—Philippine Bureau of Science parasitological collection No. 329.

Family HEMIURIDÆ Luehe, 1901

ECTENURUS LEMERIENSIS sp. nov. Plate 3, fig. 1.

This parasite is closely related to a group of flukes recently described by Yamaguti (1934) under the genus *Ectenurus* Looss, 1907, from fishes in Japan. It most closely resembles *E. hamati,* from which it may be differentiated by the shape of its testes which are oval to subglobular instead of being wedge-shaped, by the absence of a saccular swelling at the terminal portion of the uterus, and by the greater development of the vitelline glands.

Description.—Body elongate, more rounded and broader anteriorly than posteriorly; length 2.5 to 3.2 millimeters, depending upon whether the caudal appendage is extended or retracted;

maximum diameter 0.80 to 0.95 millimeter across anterior level of acetabulum. Tail in extended specimens with a maximum length of 0.42 millimeter. Cuticle unarmed. Oral sucker ventroterminal, 0.24 to 0.28 by 0.25 to 0.31 millimeter in size, with prominent preoral lip; acetabulum very well developed, 0.55 to 0.68 by 0.53 to 0.66 millimeter in size, near middle of body length, its greater bulk in front of equator of body. Prepharynx and œsophagus absent; pharynx 0.13 to 0.15 by 0.12 to 0.15 millimeter in size; intestinal cæca moderately sinuous in outline and swollen in diameter, reaching to near posterior end of tail.

Testes oval to subglobular, one obliquely behind the other and partly overlapping, antovarial and immediately behind acetabulum; anterior testis partly overlapped by acetabulum, 0.20 to 0.29 by 0.18 to 0.22 millimeter and posterior testis 0.18 to 0.33 by 0.27 millimeter in size. Seminal vesicle oval, slightly to one side of median line, at equator of body length and almost entirely overlapped by acetabulum. Pars prostatica median, about halfway between pharynx and acetabulum. Sinus sac (hermaphroditic pouch) pyriform to oval, to one side of median line, immediately behind pharynx, 0.23 to 0.28 by 0.15 to 0.19 millimeter in size. Common genital opening median to submedian, ventral to anterior level of pharynx.

Ovary in posterior third of body length, in contact with second testis, transversely oval to pear-shaped, submedian, 0.11 to 0.16 by 0.15 to 0.22 millimeter in size. Uterus with irregular coils, most of which are found behind acetabulum and the remainder anterior to that organ; the coils do not extend posteriorly beyond level of ovary. Vitellaria well developed, in coiled tubes, of which four pairs could be made out. Eggs small, thin-shelled, operculated, 15 to 17 by 9.6 to 12 microns in size.

Specific diagnosis.—Ectenurus: Total length 2.5 to 3.2 millimeters, maximum diameter 0.80 to 0.95 millimeter. Testes oval to subglobular, one obliquely behind the other and partly overlapping. Ovary transversely oval to pear-shaped, submedian, in contact with second testis. Vitellaria with four pairs of coiled tubes. Uterus does not extend posteriorly beyond level of ovary. Eggs 15 to 17 by 9.6 to 12 microns in size.

Host.—Leather jacket (*Scomberoides* sp.).

Location.—Intestine.

Locality.—Balayan Bay, Lemery, Batangas, Luzon.

Type specimens.—Philippine Bureau of Science parasitological collection No. 419.

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ILLUSTRATIONS

[Drawings by A. C. Gonzales.]

PLATE 1

FIG. 1. *Opisthorchis ophidiarum* sp. nov., entire worm, dorsal view.
2. *Echinostoma charadrii* sp. nov., entire worm, ventral view.
3. *Echinostoma charadrii* sp. nov., head collar, ventral view.

PLATE 2

FIG. 1. *Pegosomum bubulcum* sp. nov., entire worm, ventral view.
2. *Paradistomum excalotes* sp. nov., entire worm, ventral view.

PLATE 3

FIG. 1. *Ectenurus lemeriensis* sp. nov., entire worm, ventral view.
2. *Euclinostomum multicæcum* sp. nov., entire worm, ventral view.

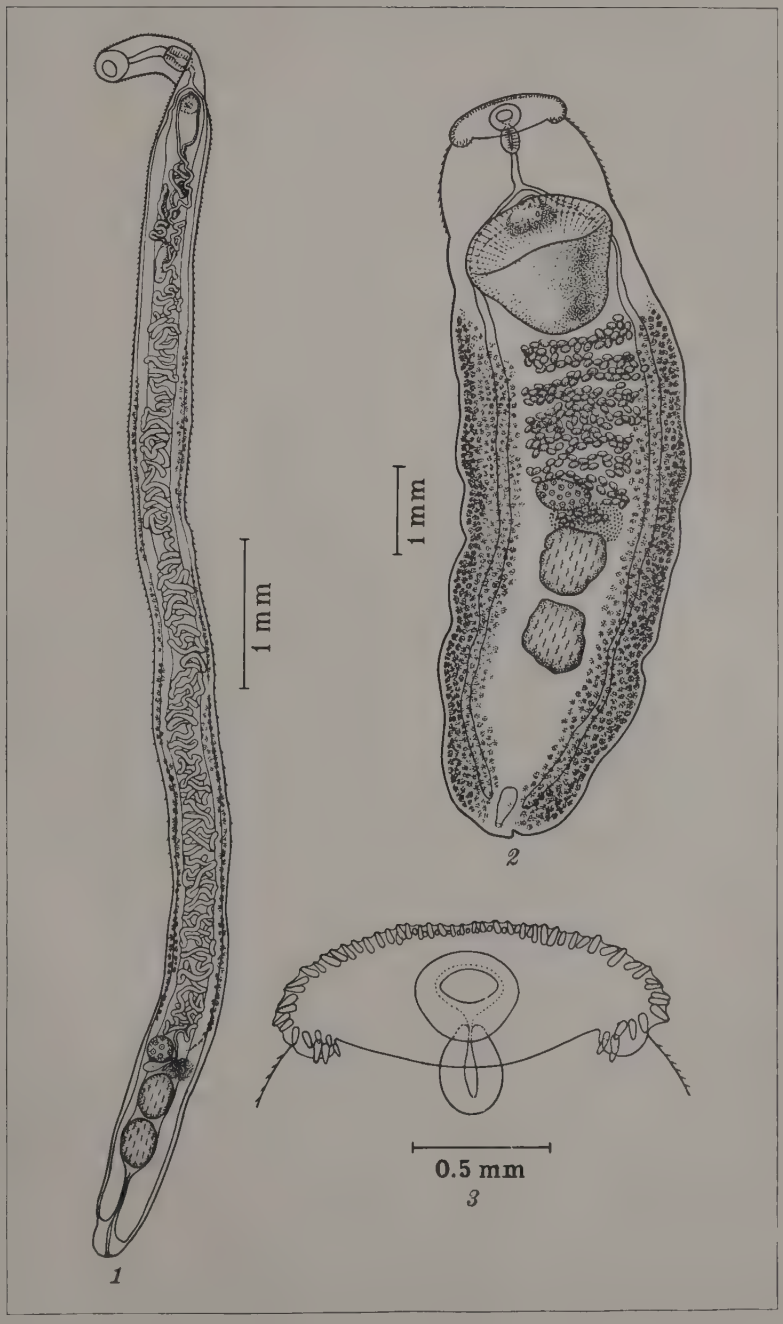

PLATE 1.

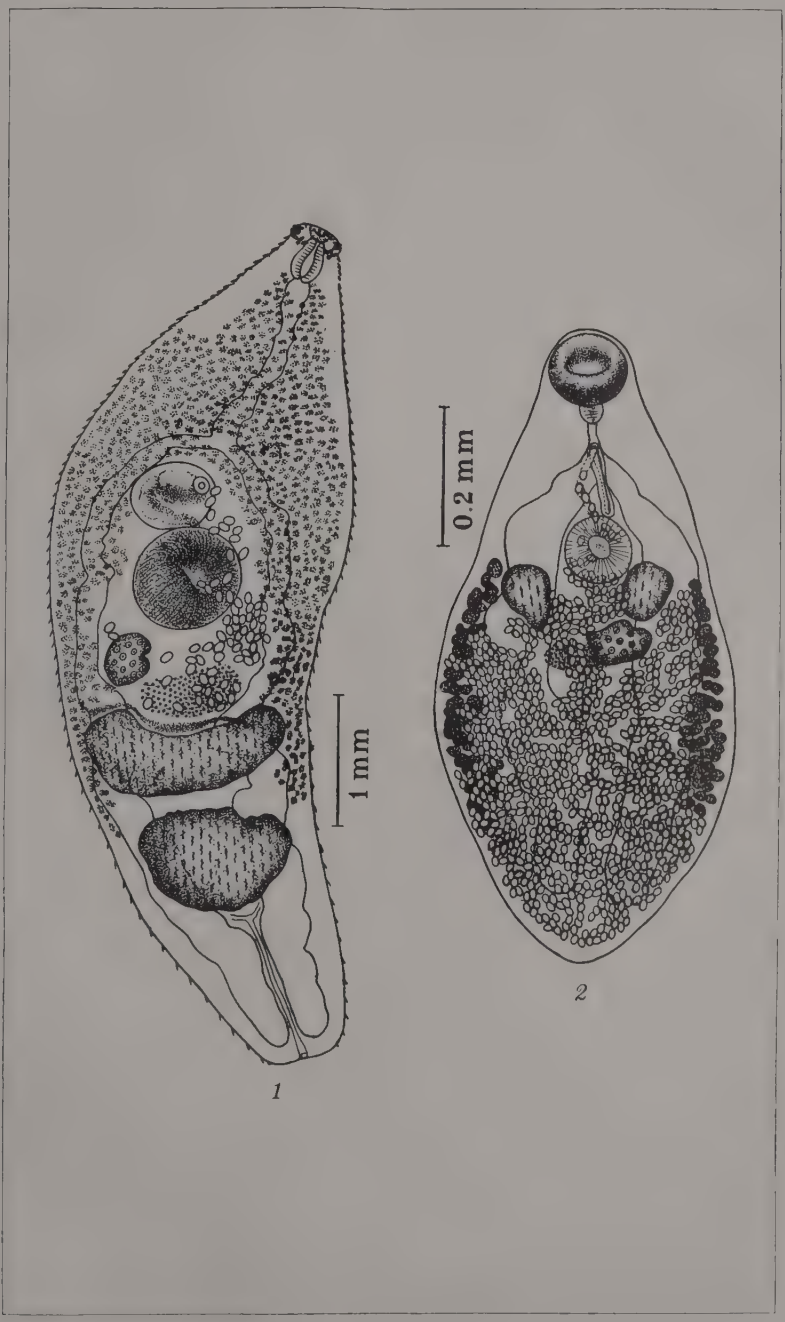

PLATE 2.

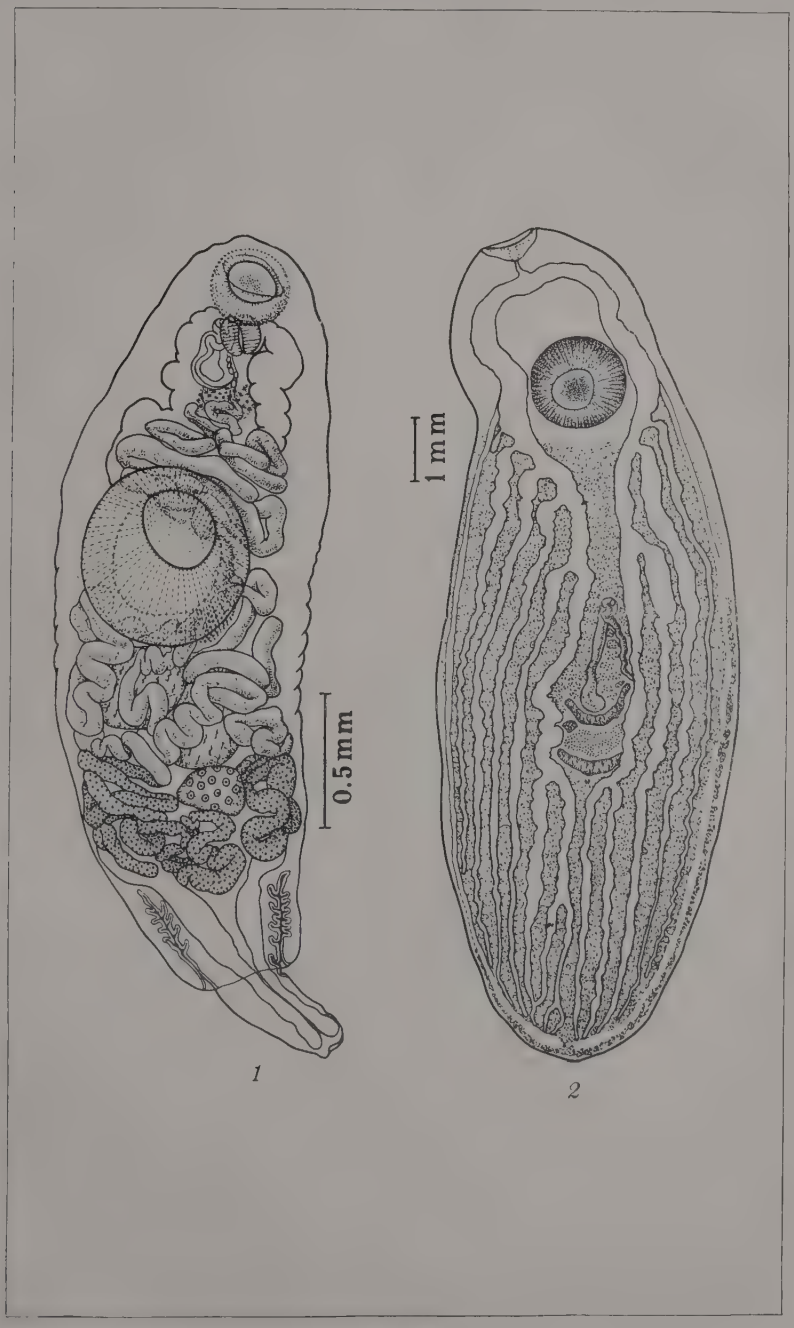

PLATE 3.

OBSERVATIONS ON THE GEOGRAPHICAL DISTRIBUTION OF HOOKWORM PARASITES AND HOOKWORM DISEASE IN THE PHILIPPINES [1]

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SEVEN TEXT FIGURES

A question in parasitology that has remained unanswered is the extent to which hookworms are a menace to public health in the Philippines. The large majority of the early workers in medical zoölogy in the Islands expressed the view that the parasites have no local medical importance, due either to the lightness of the infestations (Gomez, 1911), a racial immunity enjoyed by the Filipino people against the effects of the parasites (Schwartz and Tubangui, 1922), or the possible existence in the Philippines of a harmless variety of hookworm (Heiser, 1916).[2] On the other hand Leach, Schwartz, Leach and Haughwout (1923), as well as Manalang (1925), recorded typical cases of hookworm disease in Cebu, where, judging from the reports of these authors, the infestation is a public-health problem.

It occurred to the present writers that these varying opinions on the medical significance of hookworms in the Philippines might be due, as has been found in other parts of the world, to an uneven distribution of the parasites in the Islands, taking for granted that the prevalence or absence of hookworm disease in certain places is due to the existence of favorable conditions for the propagation of the parasites and vice versa. To verify our suspicion it was planned to carry out quantitative hookworm surveys in different parts of the Archipelago.

[1] Read in part before the Third Philippine Science Convention held in Manila February 26, 1935.

[2] According to Leach, Haughwout and Ash (1923), Manalang (1925) and Tubangui and Francisco (1925) the prevailing species of hookworm in the Philippines is *Necator americanus*.

PLACES SURVEYED AND TIME OF YEAR

The data presented in this paper are the results of observations that were made during the last four years in the following places: In Palo, Leyte, from January 27 to February 10, 1932; in Manila, from July 25 to September 23, 1932; in Pardo, Cebu, from April 10 to 30, 1933; in Paombong, Bulacan, from May 15 to June 3, 1933; in Cabuyao, Laguna, from October 16 to 21, 1933; in Baao, Camarines Sur, from April 6 to 22, 1934; in Masbate, Masbate, from September 8 to 13, 1934; in Catbalogan, Samar, from September 15 to 18, 1934; in Las Piñas, Rizal, from November 8 to December 5, 1934; and in Cotabato, Cotabato, from April 5 to 11, 1935. These places are very widely separated from one another (text fig. 7) and represent in a marked degree the various conditions of life and environment that are met with in the Islands. For these reasons it was expected that the results of the examinations would yield reliable information on the geographical distribution of hookworm parasites in the Philippines.

MATERIALS AND METHODS

The total number of persons examined was 2,357, distributed as follows: 169 in Palo, Leyte; 503 in Manila; 489 in Pardo, Cebu; 401 in Paombong, Bulacan; 116 in Cabuyao, Laguna; 220 in Baao, Camarines Sur; 56 in Masbate, Masbate; 34 in Catbalogan, Samar; 315 in Las Piñas, Rizal; and 54 in Cotabato, Cotabato. Those examined in Manila were school children from 7 to 11 years of age, while those examined in the other places represented mixed populations.

The individual fæcal samples were examined according to the modified Stoll egg-counting technic (Stoll and Hausheer, 1926) and in recording the egg counts the figures were brought to the formed basis (Stoll, 1929) and corrected for size according to age (Cort, Otto, and Spindler, 1930). For the isolation of hookworm larvæ from the soil the well-known Baermann apparatus was used, and for measuring the effects of the parasites on the hosts, hæmoglobin determinations with the Tallquist scale were made.

RESULTS OF EXAMINATIONS

Of the total number of 2,357 persons examined, 1,048, or 44.5 per cent, were found positive for hookworms, the average egg count of the entire series being 1,850 per cubic centimeter of

fæces. These figures, however, do not portray accurately the hookworm situation in the Philippines, for which reason the results of the examinations from each of the areas covered by the survey will be discussed separately and then compared with one another. Table 1 gives the incidence of the parasites and the average egg counts arranged according to population groups and analyzed according to age and sex. The age grouping is that suggested by Cort, Riley, Sweet, Schapiro, and Stoll (1929) and is as follows: 0- to 4-year group, representing the youngest children, who are largely confined to the neighborhood of their homes; 5- to 14-year group, which includes the school children and in which there are but little differences in activities between the two sexes; 15- to 49-year group, in which most of the males are engaged in field and other activities and the females in household duties and the rearing of children; 50 + year group, in which both sexes, due to advanced age, tend to be more closely confined to the house.

Pardo, Cebu.—Our hookworm egg counts from this locality represent the results of the examination of the same fæcal samples on which our egg counts for ascaris and trichuris from the same town, as described in an earlier paper (Tubangui, Basaca, and Pasco, 1934), were made. Of the 489 individuals examined, 395, or 80.9 per cent, were positive for hookworms, the incidence being 84.2 per cent in the 253 males and 77.1 per cent in the 236 females. The average egg count of the entire series was 2,750, that of the males alone 3,200 and that of the females 2,300. The index of infection, according to the method of Chandler (1928), was 191 for the whole group. It is shown in Table 1 that the very young children of both sexes are very lightly parasitized, but the children of school age have practically the same egg counts as the adults. As shown in text fig. 1, this suggests a rapid acquisition of the parasites by the children. It is also noted that the males in the various age groups are more heavily infested than the corresponding groups of females, indicating that the opportunities of picking up the parasites are greater in the males than in the females. In the males the peak of the incidence curve occurs in the 50 + group, while that of the average egg count curve is found in the 5- to 14-year group. In the females the infestation in the 5- to 14-year group is quite heavy, but the peaks of both the incidence and egg-count curves occur in the 50 + group.

TABLE 1.—*Hookworm egg counts of ten groups of individuals in the Philippines, classified according to age and sex.*

MALES.

Locality.	0 to 4 years.				5 to 14 years.				15 to 49 years.			
	Cases.	Positive.	Incidence.	Average egg count per cc.	Cases.	Positive.	Incidence.	Average egg count per cc.	Cases.	Positive.	Incidence.	Average egg count per cc.
			Per cent.				*Per cent.*				*Per cent.*	
Pardo, Cebu	16	6	37.5	400	90	78	86.7	3,800	128	111	86.7	3,000
Palo, Leyte	7	3	42.9	550	25	21	84.0	4,300	53	45	84.9	5,700
Baao, Camarines Sur	3	1	33.3	300	48	37	77.1	3,200	109	77	70.6	4,700
Cotabato, Cotabato	3	0	0.0	0	10	7	70.0	10,900	19	11	57.9	19,000
Masbate, Masbate	6	2	33.3	800	8	4	50.0	1,000	20	16	80.0	4,700
Catbalogan, Samar	0	0	0.0	0	10	10	100.0	1,800	12	10	83.3	2,000
Cabuyao, Laguna	1	0	0.0	0	10	5	50.0	1,600	33	22	66.6	1,800
Paombong, Bulacan	26	5	19.2	100	53	32	60.4	1,000	97	66	68.0	1,800
Las Piñas, Rizal	9	0	0.0	0	42	3	7.1	50	55	6	10.9	100
Manila	0	0	0.0	0	259	20	7.7	100	0	0	0.0	0
FEMALES.												
Pardo, Cebu	18	10	55.5	300	72	61	84.7	2,900	122	89	73.0	2,000
Palo, Leyte	4	1	25.0	250	23	16	69.6	1,650	45	30	66.6	3,000
Baao, Camarines Sur	4	1	25.0	100	4	2	50.0	1,600	26	13	50.0	1,100
Cotabato, Cotabato	2	0	0.0	0	9	5	55.5	5,150	9	6	66.6	12,000
Masbate, Masbate	2	0	0.0	0	6	2	33.3	1,200	10	8	80.0	2,100
Catbalogan, Samar	0	0	0.0	0	0	0	0.0	0	10	8	80.0	1,000
Cabuyao, Laguna	0	0	0.0	0	15	4	26.7	200	37	12	32.4	1,100
Paombong, Bulacan	19	4	21.0	300	52	24	46.1	500	107	29	27.1	500
Las Piñas, Rizal	9	0	0.0	0	46	0	0.0	0	115	8	6.9	20
Manila	0	0	0.0	0	244	9	3.7	50	0	0	0.0	0

MALES.

Locality.	50 years and over.				All ages,			
	Cases.	Positive.	Incidence.	Average egg count per cc.	Cases.	Positive.	Incidence.	Average egg count per cc.
			Per cent.				*Per cent.*	
Pardo, Cebu	19	18	94.7	3,600	253	213	84.2	3,200
Palo, Leyte	8	8	100.0	6,000	93	77	82.8	4,950
Baao, Camarines Sur	23	22	95.6	7,300	183	137	74.9	4,500
Cotabato, Cotabato	2	0	0.0	0	34	18	52.9	9,000
Masbate, Masbate	4	4	100.0	3,500	38	26	68.4	1,700
Catbalogan, Samar	0	0	0.0	0	22	20	90.9	1,000
Cabuyao, Laguna	11	6	54.5	800	55	33	60.0	1,600
Paombong, Bulacan	23	9	39.1	900	199	112	56.3	1,200
Las Piñas, Rizal	19	4	21.0	700	125	13	10.4	150
Manila	0	0	0.0	0	259	20	7.7	100
FEMALES.								
Pardo, Cebu	24	22	91.7	3,900	236	182	77.1	2,300
Palo, Leyte	4	3	75.0	3,300	76	50	65.8	2,500
Baao, Camarines Sur	3	1	33.3	150	37	17	45.9	1,000
Cotabato, Cotabato	0	0	0.0	0	20	11	55.0	7,700
Masbate, Masbate	0	0	0.0	0	18	10	55.5	800
Catbalogan, Samar	2	2	100.0	1,000	12	10	83.3	1,000
Cabuyao, Laguna	9	1	11.1	50	61	17	27.9	500
Paombong, Bulacan	24	8	33.3	700	202	65	32.2	500
Las Piñas, Rizal	20	0	0.0	0	190	8	4.2	10
Manila	0	0	0.0	0	244	9	3.7	50

TABLE 2.—*Average hæmoglobin percentages of Pardo series arranged according to hookworm infestation intensity groups.*

MALES.

Hookworm infestation groups in eggs per cc.	Ages 0 to 4 yrs.		Ages 5 to 14 yrs.		Ages 15+ yrs.		All ages.		
	Cases.	Mean Hb reading.	Cases.	Mean Hb reading.	Cases.	Mean Hb reading.	Cases.	Per cent of sex.	Mean Hb reading.
Negatives	10	74.0±1.77	12	75.0±1.64	18	82.9±0.84	40	15.8	78.2±0.88
100 to 999	4	68.8±2.29	11	70.0±1.84	25	79.6±0.90	40	15.8	76.0±0.93
1,000 to 4,999	2	72.5±1.20	46	72.0±0.60	74	77.8±0.66	122	48.2	75.5±0.51
5,000 to 9,999	0		8	70.0±1.94	19	73.7±1.61	27	10.7	73.6±1.41
10,000+	0		13	57.3±1.50	11	53.6±2.11	24	9.5	55.6±1.29
Total males	16		90		147		253	100.0	
FEMALES.									
Negatives	8	78.1±1.31	11	75.6±1.26	35	80.0±0.37	54	22.9	78.9±0.70
100 to 999	8	72.0±1.21	13	76.2±1.68	35	78.1±0.86	56	23.7	76.8±0.81
1,000 to 4,999	2	72.5±0.59	33	75.7±0.94	56	76.8±0.63	91	38.5	76.3±0.48
5,000 to 9,999	0		13	70.8±1.82	18	72.8±0.89	31	13.2	71.9±1.09
10,000+	0		2	42.5±3.57	2	42.5±3.57	4	1.7	42.5±2.52
Total females	18		72		146		236	100.0	
Total both sexes	34		162		293		489		

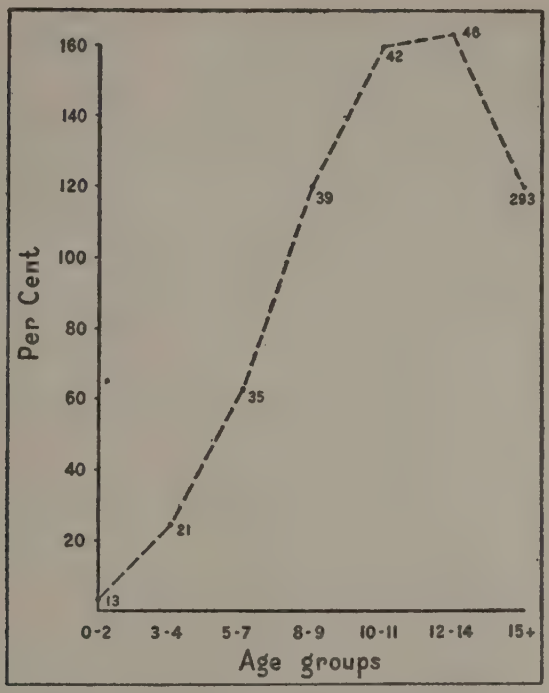

FIG. 1. A representation of the rate of acquisition of hookworm parasites in the children of Pardo, Cebu. The average egg count for each group is given as a percentage of the average count of the adults (15 + years). The figures at each point in the curve give the number of cases used in computing the value.

Table 2, in which the individual egg counts are arranged in intensity groups, shows the same age and sex differences between the infestations. Among the males 31.6 per cent and among the females 46.6 per cent fall in the negative and lowest egg-count groups, in which the estimated number of adult worms harbored, based on the factor of 100 eggs per adult worm proposed by Cort, Stoll, and Grant (1926), is less than 10. On the other hand among the most heavily infested cases the number of the males is greater than that of the females, there being 24 of the former and only 4 of the latter with counts of 10,000 + eggs per cubic centimeter of fæces. To a large extent the preponderance of these heavily infested cases among the males is responsible for the much higher average egg counts of the males as compared with the counts of the females. The

highest individual counts in the two sexes were 20,800 and 20,400 and were met with in a male 45 years old and in a female 60 years old, respectively.

Baao, Camarines Sur.—In this locality 220 persons were examined. Of the 183 males, 137, or 74.9 per cent, were hookworm positives, their average egg count being 4,500. Of the 37 females, 17, or 45.9 per cent, were infested, their average egg count being 1,000. The incidence in the entire series is thus 70 per cent and the average egg count 3,900. By comparing these figures with those of the Pardo series it will be seen that the incidence of the parasites is lower in Baao, but

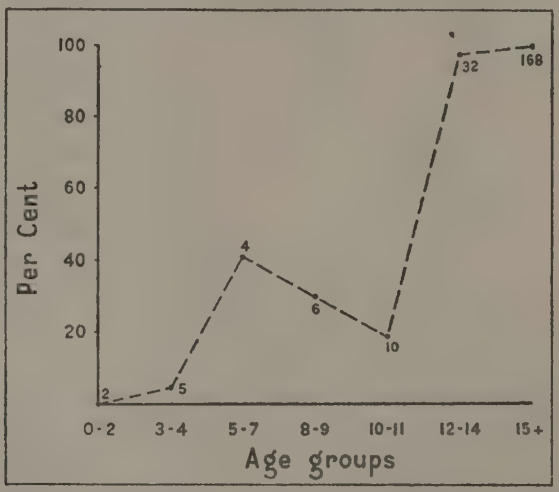

FIG. 2. A representation of the rate of acquisition of hookworm parasites in the children of Baao, Camarines Sur (method used as in text fig. 1).

the degree of infestation is more than twice as heavy. The index of infection, which was found to be 300, is much higher. As in the Pardo series, the males appear to be more heavily parasitized than the females. The analysis into age groups, however, shows a much lighter infestation in the school children, indicating a slower rate of acquisition of the parasites by the Baao children compared with the Pardo children (text fig. 2). An analysis of the counts into intensity groups shows a relatively greater number of heavily infested cases, which account to a large extent for the higher average egg count of the group. The highest individual counts for the two sexes are 102,000

and 32,000 and were encountered in a male 48 years old and in a female 32 years old, respectively.

Palo, Leyte.—The number of persons examined in this place was 169, of which 93 were males and 76 were females. The number of hookworm positives was 127, or 75.1 per cent, and the average egg count was 3,850. The incidence in the males alone was 82.8 per cent and the average egg count 4,950, while in the females the incidence was 65.8 per cent and the average egg count 2,500. The index of infection was found to be 290.

The analysis of the data according to age and sex shows that the males are about twice as heavily infested as the females and that the children of school age have more than one-half of the infestation of the adults. The severity of the infestation in this locality is indicated by the large number of individuals (24.8 per cent) found to be heavily parasitized and apparently suffering from hookworm disease.

Cotabato, Cotabato.—Only 54 individuals were examined in this place, of which 34 were males and 20 females. The males had an incidence of 52.9 per cent and an average count of 9,000 per cubic centimeter of fæces. The incidence in the females was 55 per cent and the average egg count 7,700. Considering the entire group, the incidence was 53.7 per cent and the average egg count 8,500. The degree of infestation is thus much heavier than that of either the Pardo, Baao, or Palo series, but this is probably due to the fact that the majority of those examined were adults, a large number of whom were heavily parasitized. As a matter of record the highest individual counts were 59,000 and 38,200 and were met with in a male 10 years old and in a female 17 years old, respectively. The data, although incomplete, may be taken as an indication of the importance of hookworm parasites in this locality.

Masbate, Masbate.—The number of cases examined in this place consists of only 56 individuals, of whom 36, or 73.2 per cent, were positive for hookworms. The average egg count of the entire group was 1,400. The incidence in the 38 males was 68.4 per cent and the average egg count 1,700; in the 18 females the incidence was 55.5 per cent and the average egg count 800. We had expected to find the parasites of medical importance in this locality, but the data obtained denote only a low grade of hookworm infestation. This is probably explained by the fact that the persons who submitted fæcal samples lived in or near

TABLE 3.—*Average hæmoglobin percentages of Paombong series arranged according to hookworm infestation intensity groups.*

MALES.

Hookworm infestation groups in eggs per cc.	Ages 0 to 4 yrs.		Ages 5 to 14 yrs.		Ages 15+ yrs.		All ages.		
	Cases.	Mean Hb reading.	Cases.	Mean Hb reading.	Cases.	Mean Hb reading.	Cases.	Per cent of sex.	Mean Hb reading.
Negative	21	78.9±1.50	21	82.1±1.10	45	79.5±0.58	87	43.7	80.0±0.66
100 to 999	2	77.5±1.20	13	77.7±0.79	12	77.5±0.56	27	13.6	77.5±0.67
1,000 to 4,999	3	78.3±2.64	13	80.0±0.73	40	79.1±0.40	56	28.1	79.1±0.74
5,000 to 9,999	0		6	78.3±1.45	22	80.0±1.10	28	14.1	78.6±0.87
10,000+	0		0		1	70.0	1	0.5	70.0
Total males	26		53		120		199	100.0	
FEMALES.									
Negative	15	78.6±1.92	28	77.9±1.78	94	79.4±0.70	137	67.8	78.6±0.86
100 to 999	3	78.6±2.61	11	80.0±1.60	12	76.6±2.20	26	12.9	78.7±0.86
1,000 to 4,999	1	76.6	9	78.0±0.45	19	75.7±1.30	29	14.4	76.9±0.57
5,000 to 9,999	0	77.5±1.45	4	77.5±1.48	6	76.6±2.20	10	4.9	77.3±0.81
10,000+	0		0		0		0		
Total females	19		52		131		202	100.0	
Total both sexes	45		105		251		401		

the town center, which is very close to the sea, and the people deposited their fæces near the seashore or directly into the water. Under such conditions the danger of acquiring hookworm parasites is not very great. The high average egg count of the males in the 5- to 14-year group is due to a single heavily infested case, a boy 14 years old, whose egg count was 34,000.

Catbalogan, Samar.—In spite of the small number of cases examined, we have decided to record our observations from this place in order to complete our report. Of the 34 individuals examined, the majority of whom were over 10 years of age, 30, or 88.2 per cent, were hookworm positives. The average egg count, however, was only 1,000, not a single case presenting a count of more than 5,000. It would thus appear that the infestation in Catbalogan is of the light type, but our low figures are explained by the fact that, as in Masbate, nearly all of the individuals who submitted fæcal samples lived in the poblacion, where the environment is not favorable for the propagation of hookworms due to its proximity to the seashore.

Paombong, Bulacan.—Our hookworm egg counts from this town represent the results of the examination of the fæcal samples on which our egg counts for ascaris and trichuris from the same town, as reported in an earlier paper (Tubangui, Basaca, and Pasco, 1934), were made. The group was composed of 401 individuals, of which 177, or 44.1 per cent, were found positive for hookworms, the incidence in the 199 males being 56.3 per cent and in the 202 females 32.2 per cent. The average egg count of the entire series was 850 per cubic centimeter of fæces, that of the males alone being 1,200 and that of the females 500. The analysis of the data according to age and sex shows that the infestations in the different groups are uniformly light or mild in character. As a matter of fact there was only one individual in the entire series who had an egg count of 11,600, the counts of the majority of the other hookworm positives not being over 3,000 (Table 3). These figures and the index of infection, which was only 40, denote a very low grade of hookworm infestation which, in the words of Chandler (1920), "is so small that it is of no practical consequence and can safely be ignored as an important public health problem."

Cabuyao, Laguna.—This series is composed of 116 persons, of which 50, or 43.1 per cent, were positive for hookworms, the incidence in the 55 males being 60 per cent and in the 61 females 27.9 per cent. The average egg count of the entire series was

1,020, that of the males alone 1,200 and that of the females 500. These data denote a low grade of hookworm infestation, but there were in the series five heavily infested individuals, one of whom in particular attracted our attention due to his extreme pallor (hæmoglobin reading, 40) and physical weakness. He was in other words suffering from typical hookworm disease. One of us (M. B.) treated him for hookworms and he made a rapid recovery. It would appear from these observations that Cabuyao is one of the few places in the provinces near Manila where hookworms find suitable environment for their development. As a matter of fact, the index of infection, which was calculated to be 180, differs but little from that of Pardo, Cebu.

Las Piñas, Rizal.—Of the 315 persons examined in this place, only 21, or 6.6 per cent, were positive for hookworms, the incidence in the 125 males being 10.4 per cent and in the 190 females 4.2 per cent. All of the positive cases were very lightly infested, the average egg count of the entire series being 70, that of the males alone 150, and that of the females 10. The index of infection was calculated to be only 10, showing that the hookworm infestation in Las Piñas, Rizal, can be safely overlooked as a public-health problem.

Manila.—As already stated, this series is composed entirely of school children, who were the same ones on whom our ascaris and trichuris counts, as reported in an earlier paper, were based. Of the 503 children examined, only 29, or 5.8 per cent, were positive for hookworms, the incidence in the 259 boys being 7.7 per cent and in the 244 girls 3.7 per cent. The average egg count of the entire group was only 50, the intensity of the infestation in the two sexes being the same. Comparison of these data with those of the children of the other series of individuals examined shows that parasitism in Manila is very light and therefore negligible from the public-health standpoint. As a matter of fact, the index of infection was only 10.

GEOGRAPHICAL DISTRIBUTION OF HOOKWORM PARASITES IN THE PHILIPPINES

The data given above show that the degree of hookworm infestation in the Philippines varies in the different localities. For purposes of comparison the infestations of the ten groups of individuals examined during the survey are represented graphically by means of bar diagrams in text fig. 3, in which the average egg counts of the Pardo series, classified ac-

cording to age and sex, are each given a weight of 50, and the ratio to this figure of the corresponding counts of each of the

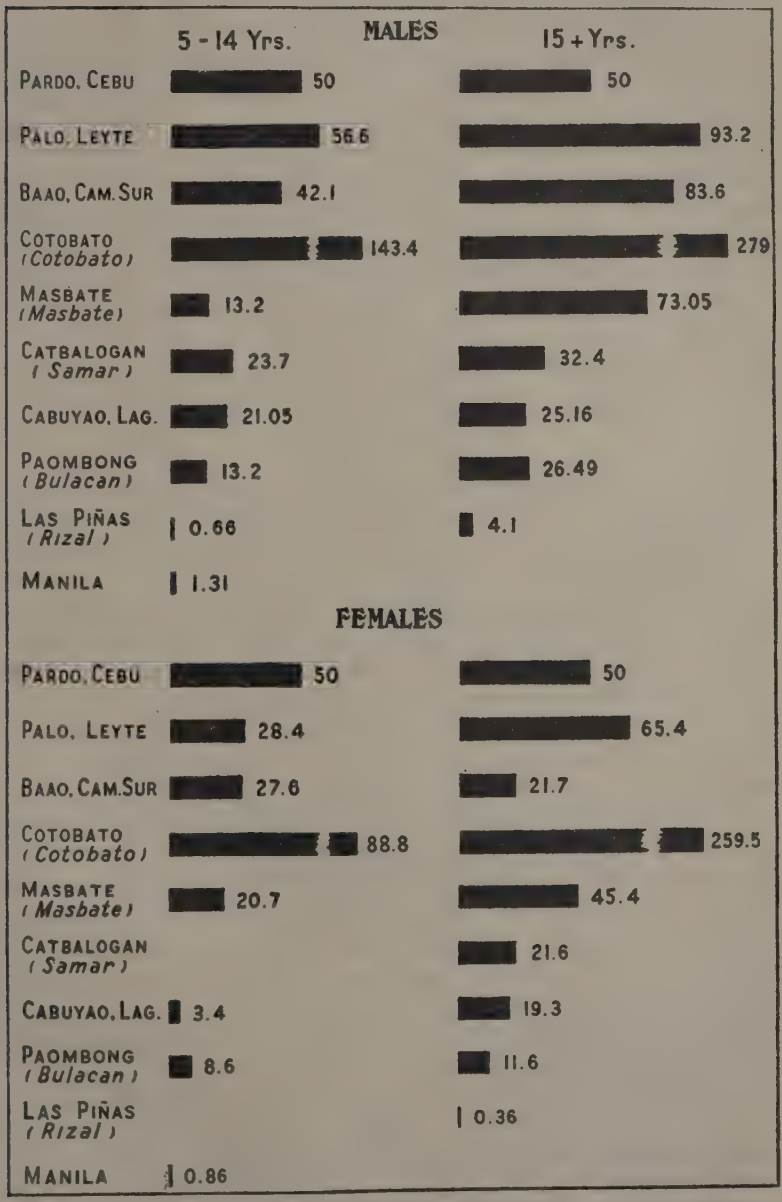

FIG. 3. A representation of the differences between the hookworm egg counts of the different series of individuals examined.

other series is then calculated. Children from 0 to 4 years of age have not been included in the comparisons, because of their uniformly very light infestations.

According to the bar diagrams the places where the infestations were found to be heaviest were Pardo, Cebu; Palo, Leyte; Baao, Camarines Sur; and Cotabato, Cotabato. The lightest infestations, on the other hand, were encountered in Manila; Las Piñas, Rizal; Paombong, Bulacan; and Cabuyao, Laguna. By obtaining the general average of the counts of the individuals from Pardo, Palo, Baao, and Cotabato, it is calculated that the intensity of the parasitism in these four localities is almost nine times as heavy as that in Manila, Las Piñas, Paombong, and Cabuyao. Our data from Masbate and Catbalogan are really too limited to be of much significance, but there are indications that in these islands, especially in the interior towns, hookworms may be found to be of public-health importance.

HOOKWORM DISEASE

In view of the skepticism regarding the medical importance of hookworms in the Philippines, we shall record our observations on the existence and prevalence of hookworm disease in the different localities covered by our survey, based on the results of our hæmoglobin determinations. We desired to utilize for this purpose all of our available data, but the occurrence in endemic form in several of the places visited of either malaria or schistosomiasis japonica or both, which, like hookworm disease, are characterized by anæmia, rendered the interpretation of many of our hæmoglobin averages difficult. Although the Uncinariasis Commission to the Orient (Darling, Barber, and Hacker, 1920) concluded from its findings that the hæmoglobin index is a reliable guide in estimating the severity of hookworm disease irrespective of other anæmia-producing conditions, we have decided to present only the data obtained in Pardo and Paombong in order to exclude from the discussion these complicating factors. As far as we were able to ascertain both by direct observation and inquiry from private and public-health physicians, only these two towns were free endemically from malaria and schistosomiasis.

Tables 2 and 3 give the hæmoglobin averages of the Pardo and Paombong series, analyzed according to age and sex and lined up for the various hookworm intensity groups. It is evident from the figures that a definite correlation exists between the

hæmoglobin index and the number of parasites harbored, the hæmoglobin readings being usually lower in the higher egg count groups. In the Paombong series none of the hæmoglobin averages is below 70, due to the lightness of the infestations, but in the Pardo series they are very reduced in the group of the heavily infested individuals. In the latter series, as shown graphically

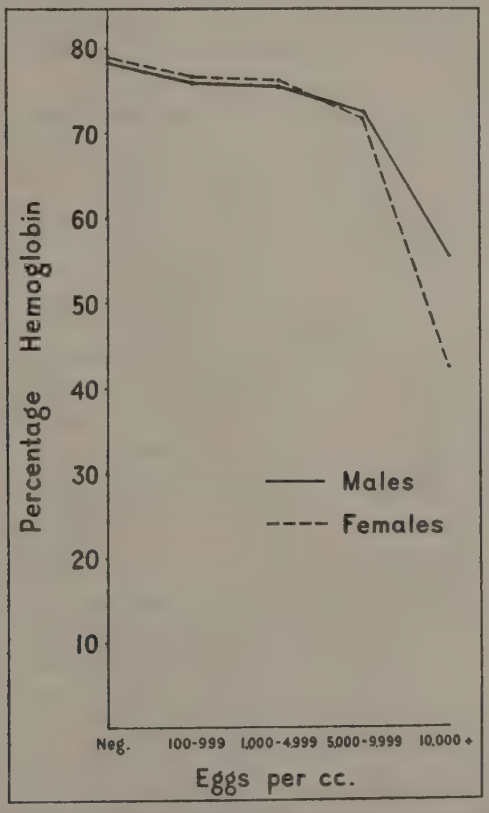

FIG. 4. Hæmoglobin averages of Pardo series as related to hookworm intensity groups.

in text fig. 4, the hæmoglobin curve falls very gradually to 72.6 ± 1.31 in the males and 71.9 ± 1.09 in the females in the 5,000 to 9,999 egg-count groups, and then abruptly to 55.6 ± 1.29 in the males and 42.5 ± 2.57 in the females in the 10,000 + groups of the two sexes. These hæmoglobin averages seem to suggest that in Filipinos the defenses of the body against the toxic effects

of hookworms are capable of holding their ground in moderately heavy infestations, in which the number of parasites harbored is less than 100. They tally with the findings of Darling, Barber, and Hacker (1920) in Java, where it was noted "that the losses (hæmoglobin) due to hookworm disease were markedly apparent only after the 100-worm group had been passed." According to the factor proposed by Cort and his associates (1926) in estimating the number of adult worms from egg counts, our 10,000 + egg-count group would include individuals harboring 100 + adult parasites.

Our findings thus prove the claim of Leach and his associates (1923) of the existence of hookworm disease as a clinical entity in the Philippines. As a public-health problem, however, the disease is not evenly distributed, being more prevalent in places where the danger of acquiring heavy hookworm infestation is great due to favorable environmental conditions. To our mind this uneven distribution of the parasites serves to explain the failure of earlier investigators in the Islands to find cases of hookworm disease; for it will be recalled that the majority of them made their observations in Manila and in some of the neighboring provinces where, according to our data, the infestations are light and apparently of no medical importance.

FACTORS RESPONSIBLE FOR THE DISTRIBUTION OF HOOKWORM PARASITES AND DISEASE IN THE PHILIPPINES

Some of the earlier investigators observed slight differences in the geographical distribution of hookworm parasites in the Philippines and proposed various explanations to account for the differences. Rissler and Gomez (1909) noted that "at Las Piñas where the soil was sandy the infection was found to be greater than in the places where the soil was of clay loam." This is in contrast to one of the explanations given by Stitt (1911), who ascribed the low incidence of the parasites in Cavite Province to the uneven distribution of the annual rainfall and to the sandy nature of the soil. Rissler and Gomez (1910) and Willets (1911) found hookworms to be more prevalent in Cagayan Valley than in the other sections of the country where surveys had been made due, according to Willets, to the favorable character of the soil, the shade afforded by tobacco plants, and the occupation of the people.

As pointed out by Cort, Stoll, and Grant (1929), the factors that influence the spread of hookworm parasites in any region are

the climate of the region and the habits of the people. Since these factors are intimately linked with the manner of development of hookworms, it will be necessary to review briefly the life cycle of the parasites. The adult worms live in the small intestines of man, and the eggs laid by the female parasites reach the outside world with the fæces. In places where improper methods of fæcal disposal are practiced, the ova are spread on the ground where, if the environment is sufficiently warm and moist, they hatch and later develop into infective larvæ. These larvæ gain entrance into the human system usually through the bare skin of the feet and other exposed parts of the body. For this reason

FIG. 5. A representation of the factors involved in the spread of hookworm infestation. (From Cort, 1922.)

people who habitually go about with shoes and other footwear are usually free from hookworms.

The manner in which the factors mentioned above are involved in the propagation of hookworms is represented graphically in text fig. 5. The attempt to correlate these factors with the results of our egg counts showed that the uneven distribution of the parasites and the disease cannot be attributed to differences in the habits of the people, for in all of the places visited evidences of pollution of the soil with human fæces due to primitive methods of fæcal disposal were encountered and the majority

of the individuals examined went habitually barefooted and were mostly engaged in occupations that exposed them to the parasites. Therefore, there remains to be considered the environmental factor, to which we believe, is due the unequal distribution of the parasitism.

Under environment the two most important epidemiological factors are soil temperature and soil humidity; for, as already stated, hookworm larvæ have certain optimum requirements of heat and moisture for their development. These in turn are controlled by two agencies in warm countries: primarily, by the amount and distribution of the annual rainfall; and, secondarily, by the presence and nature of the vegetation.

Regarding the first agency, the average annual rainfall in the Philippines, according to Father Coronas, is 2,366.1 millimeters, the annual mean in any single place varying between 989.8 and 4,597.6 millimeters. The yearly precipitation in the Archipelago thus compares favorably with that in other countries where hookworms are a major public-health problem. According to Father Coronas, however, "the most interesting feature of the climate of the Philippines is the monthly distribution of rainfall. If this element would be about the same throughout the Archipelago, there would hardly be any difference of climate in the Philippines. But as it is, the different position of the islands which makes them or part of them more or less exposed to the general winds prevailing in the Philippines, both in winter and in summer, is the principal cause of our different kinds of climate in spite of the relatively small extension of the Archipelago from east to west, especially in Luzon . . . Based on these observations, and taking in consideration the greater or less prevalence of either of the two most important periods of rain, we have tried to divide this monthly distribution of rainfall into four types: two altogether opposite types and two other intermediate types." These types of climate are as follows: First type, in which there are two pronounced seasons, dry in winter and spring, wet in summer and autumn; second type, in which there is no dry season, with a very pronounced maximum rain period in winter; third, or intermediate A, type, in which there is no very pronounced maximum rain period but only a short dry season lasting from one to three months; and fourth, or intermediate B, type, in which there is no very pronounced maximum rain period and no dry season. These four types of monthly distribution of rainfall are illustrated graphically in text fig. 6

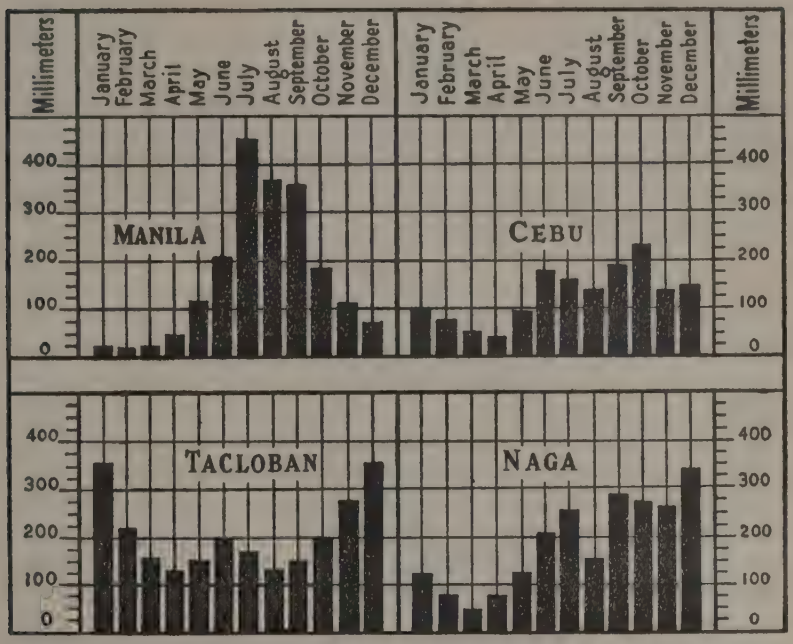

FIG. 6. Four types of monthly distribution of the annual rainfall in the Philippines. (From Coronas, 1920.)

and the different places in the Islands where they are met with are shown in text fig. 7.

The influence of these four types of climate on the temperature and on the moisture content of the soil needs hardly to be discussed, for it can be easily understood that other things being equal, the warmth and the humidity of the ground will be more constant in places where rains are more frequent than in localities where there are two pronounced seasons. With regard to the influence of the vegetation, it is well known that shaded areas are cooler and retain their moisture content longer than barren or exposed areas due to the rôle of trees and other plants in preventing the rapid evaporation of the soil moisture.

From these observations it is clear why hookworm parasites should be unevenly distributed in the Philippines. In places like Manila, Bulacan, Rizal, and Laguna, where the ground is either too wet or too dry during the greater part of the year for the normal development of hookworm larvæ due to the existence of two pronounced seasons, the infestations in the majority of cases are light and of little or no medical importance. On the

other hand, in places like Cebu, Leyte, Camarines Sur, Cotabato, and possibly in Samar and Masbate, where the temperature and humidity of the soil are more constant due to the more uniform distribution of the annual rainfall and the presence of large numbers of coconut trees in the neighborhood of the houses, the environment is favorable for the propagation of the parasites. In such places the danger of acquiring heavy hookworm infestations and of developing hookworm disease is great.

SUMMARY

In order to find a plausible explanation for the varying opinions regarding the medical significance of hookworm infestations in the Philippines, a quantitative hookworm survey was carried out in several widely separated places in the Islands.

A total of 2,357 persons were examined, distributed as follows: 169 in Palo, Leyte; 503 in Manila; 489 in Pardo, Cebu; 401 in Paombong, Bulacan; 116 in Cabuyao, Laguna; 220 in Baao, Camarines Sur; 56 in Masbate, Masbate; 34 in Catbalogan, Samar; 315 in Las Piñas, Rizal; and 54 in Cotabato, Cotabato. Those examined in Manila were school children from 7 to 11 years of age, while those examined in the other places represented mixed populations.

The egg counts showed an uneven distribution of hookworm infestation, both the incidence and the intensity of the parasitism being much higher in the groups from Cebu, Leyte, Cotabato, and Camarines Sur.

It was also noted that hookworm disease, as judged from reduced hæmoglobin percentages, was more prevalent in these four places and was invariably met with in heavily infested individuals having egg counts of 10,000 + per cubic centimeter of fæces.

The differences between the infestations could not be attributed to differences in the habits of the people examined, for in all of the places visited, with the exception of Manila, the methods of waste disposal were of the primitive type and the large majority of the people went habitually barefooted and were mostly engaged in occupations which exposed them to hookworms.

The differences were believed to be due to climatic and other environmental factors that influence the temperature and the moisture content of the soil. The fact was brought out that the distribution of the annual rainfall in the Philippines varies in

CLIMATE MAP
OF THE PHILIPPINE ISLANDS
SHOWING
SEASONS AND RAINFALL

• SEASONS •

1st Type – *Two pronounced seasons, dry in winter and spring, wet in summer and autumn.*

2d Type – *No dry season with a very pronounced maximum rainfall in winter.*

Intermediate A Type – *No very pronounced maximum rain period with a short dry season lasting only from one to three months.*

Intermediate B Type – *No very pronounced maximum rain period and no dry season.*

• RAINFALL •

The numbers o 1,963 *indicate the stations and the annual average in millimeters.*

118° 120° 122° 124° 126°

20° 18° 16° 14° 12° 10° 8° 6°

3,093 2,130 3,104 2,861 1,917 2,692 1,683 2,496 1,186 2,847 4,598 2,501 3,291 1,910 3,744 1,755 1,638 3,205 2,696 1,963 2,478 1,872 2,262 2,718 1,524 1,847 3,116 2,125 2,130 2,641 2,676 2,109 2,210 2,845 2,173 1,530 2,216 1,491 1,371 2,099 1,660 1,354 990 2,272 2,290 2,432 1,817 1,983 3,669 2,254 2,784 3,109 2,687 3,186 1,713 2,473 3,082 3,949 2,509 2,195 3,513 1,285 2,505 3,184 2,152

PAOMBONG
MANILA
LAS PIÑAS
CABUYAO
BAAO
MASBATE
PALO
CATBALOGAN
PARDO
COTABATO

Fig. 7. Climate and weather map of the Philippines, showing the various places covered by the survey. (Adapted from Coronas, 1920.)

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different regions. In some localities there are two pronounced seasons, one-half of the year being dry and the other half wet; while in other places the rains are more uniformly distributed and there is either no dry season or only a short one.

In places like Cebu, Leyte, Cotabato, and Camarines Sur, where the soil is sufficiently warm and moist during the greater part of the year due to a more or less uniform distribution of the annual rainfall and the presence in the neighborhood of human habitations of a covering vegetation that prevents the too rapid evaporation of water from the ground, the environment was considered favorable for the development of hookworm larvæ. This would explain the prevalence of heavy hookworm infestations and disease in these localities.

In places like Manila, Bulacan, Rizal, and Laguna, where the soil is either too wet or too dry during the greater part of the year due to the existence of two pronounced seasons and the absence of covering vegetations, the environment is not favorable for the spread of hookworms. This would explain why the infestations encountered in such places were light and apparently of no public-health importance.

It was concluded that the early workers in medical zoölogy underestimated the importance of hookworms in the Philippines due to the fact that the majority of them carried out their observations in Manila and in the neighboring provinces where, as stated above, the infestations appear to be very light and of no clinical significance.

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ILLUSTRATIONS

TEXT FIGURES

FIG. 1. A representation of the rate of acquisition of hookworm parasites in the children of Pardo, Cebu. The average egg count for each group is given as a percentage of the average count of the adults (15 + years). The figures at each point in the curve give the number of cases used in computing the value.

2. A representation of the rate of acquisition of hookworm parasites in the children of Baao, Camarines Sur (method as in text fig. 1).

3. A representation of the differences between the hookworm egg counts of the different series of individuals examined.

4. Hæmoglobin averages of Pardo series as related to hookworm intensity groups.

5. A representation of the factors involved in the spread of hookworm infestation. (From Cort, 1922.)

6. Four types of monthly distribution of the annual rainfall in the Philippines. (From Coronas, 1920.)

7. Climate and weather map of the Philippines, showing the various places covered by the survey. (Adapted from Coronas, 1920.)

THE PURIFICATION OF ANTIDYSENTERIC SERUM WITH SODIUM SULPHATE

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ONE PLATE

As early as 1888 Hofmeister studied the precipitating qualities of sodium sulphate with protein substances, and Pinkus (1901) employed the same salt for the precipitation of various kinds of serum albumins. The latter author showed that the precipitated proteids contained little salt (about 5 per cent), the concentration of which was found to be practically nontoxic. Brunner and Pinkus (1907) employed sodium sulphate in the separation of diphtheria antitoxin by allowing the salt to crystallize out in the refrigerator and then thawing the concentrated antitoxin. The diphtheria antitoxin recovered by this method was found to be very potent, the liquid containing but 6 per cent of sodium sulphate. In later years the salt has been used as a globulin precipitant in the determination of blood proteins (Howe, 1921) and in the study of the antigenic properties of the fractional precipitated proteins of serum (Pirowsky, 1933). Felton (1928) found this salt very convenient and useful in the concentration of pneumococcus antibody, and Maitra et al. (1933), in the concentration of antivenomous serum. The concentration with sodium sulphate of antidysenteric serum as well as antitetanic and antidiphtheritic sera was independently studied by Homer (1920) and by MacConkey (1924). The amount of sodium sulphate sufficient to salt out the different proteids of normal and immune sera was extensively discussed by the workers cited above.

Various authors have studied the sensitizing properties of the fractional proteids prepared from the serum of different animals, especially horse serum. Dale and Hartley (1916) remarked that, for the purpose of reducing serum reactions, the elimination of albumin seems to be as important as that of euglobulin when the pseudoglobulin is the fraction carrying the therapeutic power. Their observation of the relatively long

latent period of the sensitiveness to albumin is also suggestive in connection with the successive crops of serum rash that have been recorded in certain patients as the result of one injection of a serum.

The main purpose of this work has been to get rid of most of the seroalbumin in antidysenteric serum and still be able to recover a concentrated, or rather purified but potent, serum.

METHOD EMPLOYED

The following procedure has been tried with several samples of antidysenteric serum. The serum is diluted with an equal amount of distilled water and warmed in the incubator at 40° C. for about seven hours. At the same time an amount of anhydrous sodium sulphate equal in grams to 20 per cent of the total volume of the diluted serum is also warmed in the incubator. After seven hours the dry sodium sulphate is added to the diluted serum with thorough shaking. The solution is allowed to stand overnight in the incubator at 40° C. and filtered inside the incubator through thick filter paper (Whatman No. 50) the following morning. The clear filtrate, consisting of seroalbumin and salt, has a dark amber color. This filtrate is discarded. The precipitate is washed with 20 per cent aqueous solution of sodium sulphate, filling the funnel twice. The last washing is conveniently started at 4 o'clock in the afternoon so as to allow the liquid to drip overnight inside the incubator. The following morning the precipitate appears in a whitish pasty form. The filter paper with the precipitate is then carefully removed from the funnel and folded in two. The edges of the paper are likewise folded so as to prevent the precipitate from running out. The precipitate is then dried by placing it between several sheets of ordinary filter paper (coarse, German make) in a press for twenty-four hours. After that time some of the sodium sulphate will be seen to crystallize out around the edges of the filter paper and the precipitate will appear as a whitish semisolid cake. With the aid of a spatula the precipitate is separated from the filter paper and transferred into a flask. It is then redissolved in a sufficient quantity of sterile distilled water to bring up the volume equal to 20 per cent of the original volume of the serum. Sodium carbonate in an amount equal to 3 per cent of the weight of the precipitate may be added to the solution in order to facilitate the dialysis which follows. Before the solution is placed in the dialyzing bag, chloroform is added,

using 0.5 cc of the latter for the precipitate collected from 1 liter of serum. The bag is then tied to, and partly immersed in, a dialyzing tank, the temperature of the water of which is maintained at about 20° C. by means of an ice-box arrangement in which the tank is inclosed (Plate 1). In order to prevent the growth of fungi on the parchment, a sack containing crystals of thymol is submerged in the dialyzing tank. The thymol could probably be omitted, for the water is fairly cool and the period of dialysis is relatively short, being only three days. Twenty kilos of ordinary ice has been found sufficient to maintain the temperature in the dialyzing tank for twenty-four hours. To the dialysate sodium chloride is added in the proportion of 1 gram salt to each liter of the dialysate. As a preservative phenol is added in the amount of 0.5 per cent, and the dialysate is then passed through a Seitz filter.

The procedure as described above is not very complicated, but precautions should be observed that the salting of the serum be carried on at a temperature not lower than 35° C. and not higher than 40° C. The water in the tank should be maintained at a temperature between 18° and 23° C. by letting melted ice flow directly into the tank. If it happens that the temperature goes below 18° C., the water in the tank may be allowed to run for some time by turning the faucet for the outflow. The addition of phenol as preservative to the serum before the process of purification has been observed to retard filtration.

TABLE 1.—*The weight of pressed globulin precipitate and the amount of sodium sulphate before and after dialysis.*

Antidysenteric serum (sample No.).	Method used.[a]	Weight of cake per liter serum.	Sodium sulphate.	
			Before dialysis.	After dialysis.
		g.	*Per cent.*	*Per cent.*
I	Regular, salted 20 per cent	138.8		
II (with toxoid)	do	191.2		
III (lot 12)	Regular, salted 18 per cent	223.05		
IV	Thawed till no crystallization		3.99	0.108
V	do		4.08	
VI	Regular, salted 20 per cent			0.12
VII	do			0.062

[a] "Regular," means that the purified serum was directly dialyzed; "thawed," means that the serum after redissolving the cake was subjected to alternate freezing and thawing until no more crystals formed in the last freezing.

The volume of purified serum obtained according to our method is about two-thirds the original volume of the serum, and the amount of sodium sulphate present has been found to range from 0.062 to 0.12 per cent (Table 1).

PROTECTION TESTS

The potency of the purified serum was tested, using mice weighing from 18 to 20 grams. The toxin employed in the tests was selected from three broth cultures that were separately inoculated with three strains of *B. dysenteriæ* (Shiga) and incubated for two weeks. As shown in Table 2 the toxicity of these cultures was found to be practically the same, for which reason only the toxin of one strain of organism (Shiga–15) was used in all the protection tests. The results of the tests, as recorded in Table 3, show that the antidysenteric serum to which

TABLE 2.—*The toxicity of three broth toxins of B. dysenteriæ (Shiga) by intravenous injections.*

Broth toxin.	Amount injected.	Mice injected May 4, 1935.	Dates of death.		
			May 5.	May 6.	May 8, 1935.
	cc.				
Shiga–15	0.2	2	1	------	1
Do	0.5	2	2	------	------
Shiga–16	0.2	2	1	------	1
Do	0.5	2	1	1	------
Shiga–18	0.2	2	------	1	1
Do	0.5	2	1	1	------

soda was added before dialysis has higher protective properties than the serum that was dialyzed without soda. The antitoxic properties of the former compare favorably with those of the untreated original antidysenteric serum. The serum designated as lot 12 in Table 3 was produced according to our regular method of immunization. This was purified and dialyzed with soda and was found to be just as potent as that produced with formalized toxin and bacteria. Our experience with the latter method of immunization, however, is limited to the use of only three horses so that we are not yet in a position to give an opinion as to its advantages over other methods of immunization.

DISCUSSION

An inquiry into the methods of producing antidysenteric serum has been discussed and reported by Schöbl (1925). The method, the amount of salt in the purified antidysenteric serum

described in this paper represents a slight modification of that used by Brunner and Pinkus in the concentration of diphtheria antitoxin with sodium sulphate. By following the original method, the amount of salt in the purified antidysenteric serum after repeated freezing and thawing ranged from 3.99 to 4.08 per cent, and this made the filtration of the serum difficult due to the concentration of salt and gelatinous proteids which clogged the pores of the filter. Besides this difficulty the filtrate was found to have lost much of its immunologic properties. Instead therefore of freezing and thawing the serum, the precipitate was dissolved in water and immediately dialyzed for not more than three days. By this method the serum after dialysis contained only an average of 0.12 per cent salt, and it filtered easily through a Seitz filter. In one performance one Seitz filter yielded about 300 cc of filtrate in three to four hours. The serum thus prepared possessed high protective properties and compared favorably in potency with the original unconcentrated serum, as shown by the results of the protection tests already referred to (Table 3). In the first protection test, in which sample II–*a*

TABLE 3.—*Protection tests performed intravenously with controls.*

Shiga-toxin No. 15 (test dose).	Amount of pure serum.	Mice injected.	First test.		Second test.		Kind of serum.
			Living.	Dead.	Living.	Dead.	
cc.	*cc.*						
	Sample II–*a*: [a]						
0.5	0.00125	2	2	0	2	0	Purified, dialyzed with soda.
0.5	0.0025	2	1	1	2	0	
0.5	0.005	2	2	0	2	0	
0.5	0.015	2	2	0	2	0	
	Sample II–*b*:						
0.5	0.00125	2	0	2	------	------	Purified, dialyzed without soda.
0.5	0.0025	2	2	0	------	------	
0.5	0.005	2	2	0	------	------	
0.5	0.015	2	2	0	------	------	
	Sample (lot 12):						
0.5	0.00125	2	2	0	------	------	Purified, dialyzed with soda.
0.5	0.0025	2	2	0	------	------	
0.5	0.005	2	2	0	------	------	
0.5	0.015	2	2	0	------	------	
	Untreated original sample II:						
0.5	0.00125	2	2	0	------	------	Control serum.
0.5	0.0025	2	2	0	------	------	
0.5	0.005	2	2	0	------	------	
0.5	0.015	2	2	0	------	------	
	Control toxin alone:						
0.5	0.0	6	0	6	------	------	Control toxin.

[a] Samples II, II–*a*, and II–*b* were of the same batch of serum.

serum was used, one of the animals that was injected with 0.0025 cc of purified serum was found dead the day following inoculation. Repeating the same experiment, a series of mice was injected with the same sample II–*a*. The animals in this second test survived and served to check the previous one. No comment will be offered with regard to the results of sample II–*b*, another purified serum dialyzed without soda, until more experiments have been done. All animals used in this experiment were observed for twenty-five days, and the deaths recorded in this report occurred within seven days.

SUMMARY

A method of purifying antidysenteric serum by the use of sodium sulphate is described. The amount of product recovered by this method is about two-thirds the original volume of plasma or serum. The purified serum is practically free from seroalbumin and compares favorably in immunologic properties with unconcentrated antidysenteric serum.

ACKNOWLEDGMENT

We sincerely express our gratitude to Dr. Otto Schöbl, former chief of the division of biological products, Bureau of Science, who with Dr. Rita Villaamil started this work that was continued by us. We are also greatly indebted to the division of tests and standards of this bureau for the quantitative determination of sodium sulphate in our purified serum samples.

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ILLUSTRATION

PLATE 1

An ice box. *1*, Zinc receptacle where the ice is placed; *2*, tube for the flow of melted ice into the tank; *3*, dialyzing tank; *4*, dialyzing bag in operation; *5*, faucet for adjusting the temperature and the outflow of water; *6*, sack containing thymol. Dimensions of the ice box: Length, 117 cm; width, 80 cm; height, 102 cm.

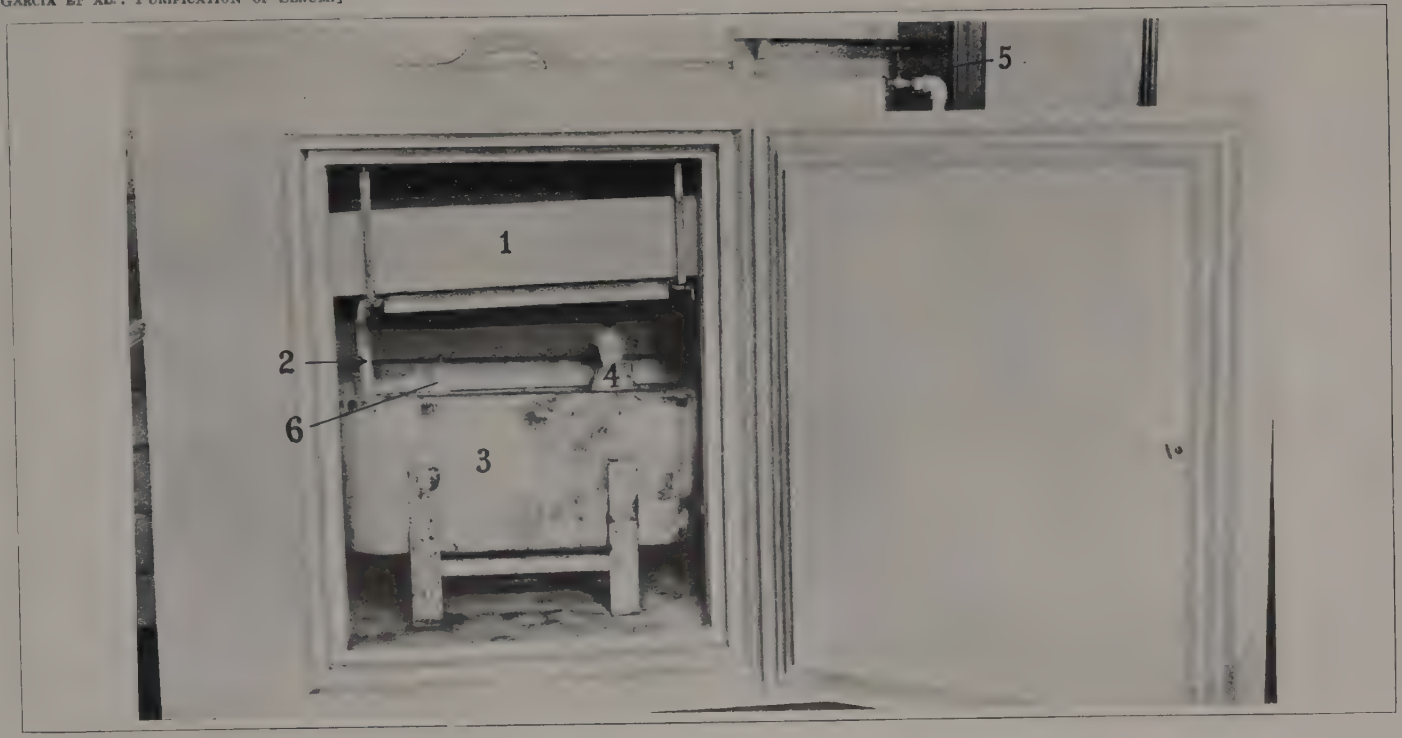

PLATE 1.

PINEAPPLE YELLOW-SPOT IN THE PHILIPPINES [1]

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FOUR PLATES

INTRODUCTION

Pineapple yellow-spot is a virosis of pineapple hitherto unreported in the Philippines. It is next in importance to bacterial fruitlet black-rot(8) and mealy-bug wilt(9) and, like the latter two, affects only the Smooth Cayenne variety up to the present. It was first noticed during the middle of 1928, in the trial plantings of the Philippine Packing Corporation in Makar, Cotabato Province, and in Santa Fe, Bukidnon Province. Owing to the characteristic stunting and dwarfing of the severely infected plants, it was at first considered as a bunchy-top disease similar to that of abacá reported by Ocfemia.(7) A careful perusal of its incipient stage has revealed, however, that its most characteristic initial symptom is the unique yellow spotting on the basal part of young leaves, hence, the name "yellow-spot," which appears to be identical with the "yellow-spot" of pineapple in Hawaii, as reported by Illingworth(1) and Linford.(4)

The yellow-spot of pineapple was first reported in Hawaii, in September, 1931, by Illingworth(1) who gives credit to Dr. C. P. Sideris as the investigator to have made in March, 1927, the first studies on the subject which, because of the characteristic rotting of one side of the affected plant, was named "side-rot;" and, to Mr. Glenn E. Paxton, for his suggesting in April, 1929, that side-rot might be a virus disease. Credit belongs to Linford(4) who, in 1931, set forth in a preliminary report his findings that the most important vector, if not the only one, operating in the field occurrence of this disease, is the onion thrips, *Thrips*

[1] This paper was not submitted for publication as early as desired because of other more pressing phytopathological problems which consumed the writer's full time.

[2] The writer is grateful to the Philippine Packing Corporation for the splendid coöperation extended him in supplying practically all the materials and labor used in the experiments, and to Dr. G. O. Ocfemia, of the Agricultural College, Laguna, for reading the manuscript.

tabaci Lindeman. A more detailed account of his report(5) was published in April, 1932.

The origin of this disease in the Philippines is not known. It is quite probable, however, that it was introduced from Hawaii through the importation of infected plant material. This view seems to find support in the fact that yellow-spot is found only among Smooth Cayenne plants imported from Hawaii.

DESCRIPTION OF THE DISEASE

The disease is manifested first by a slightly raised circular yellowish spot, the so-called "initial spot," on the upper surface of a young tender leaf (Plate 1, fig. 1). It varies in size, ranging from 2 to 15 millimeters in diameter. All the evidence shows that infection takes place somewhere near the base of a very tender leaf, but owing to the continued basal growth its first appearance is usually observed at about 8 centimeters from the base of the leaf in the third or fourth whorl. Ordinarily only one leaf shows the initial spot, although two or more have been observed with the same infection in some instances. Below the initial spot a yellow streak develops after a few days, extending down the base in the form of a water-soaked spot (Plate 1, fig. 2), and under favorable conditions rot soon follows (Plate 1, fig. 3).

In a few days more the yellow streak may show up extending to the leaf immediately above the first affected leaf, thence to all the central leaves. These leaves become stunted, chlorotic, and somewhat brittle and tend to tighten together, while all the lower leaves remain apparently normal (Plate 2, fig. 2). When cut lengthwise, diseased plants reveal patches of browned tissue in the stem around the attachment of the leaf which had the initial spot. At this point the plant evidently ceases to grow and shows a tendency to bend downward, owing to the force of growth of the opposite side (Plate 3).

Infection may take place any time during the life of the plant, although it has been observed more frequently among younger plants. Early infections prevent the plant from normal development, causing stunting, dwarfing, and ultimate death long before maturity (Plate 2, fig. 1). Late infections usually are confined to fruits and crowns. The fruits may be infected through one or more fruitlets of very tender age. This may happen during the blooming period. Infection may start from a flower or a young fruitlet, in the same manner as in a young leaf, or the crown of a young fruit. Malformation, dwarfing,

yellowing, and rotting of a few "eyes" of one side of the fruit result from this type of infection, while the opposite side develops normally, forcing the fruit to bend towards the focus of infection. In cases of advanced infection the fruit may become hollow (Plate 4). Such infected fruits resemble in every respect typical specimens of fruits affected by the so-called "Kauai disease" in Hawaii.

All kinds of planting materials have shown susceptibility to infection. It has been observed, however, that suckers are least affected and crowns most. The tightly set leaves of the former in contrast with the loosely set leaves of the latter seem to account for this fact.

WHAT CAUSES YELLOW-SPOT

The characteristic stunting, dwarfing, and chlorotic nature of the affected leaves make the diagnosis of the disease less difficult. From these symptoms it was believed that the malady is of a virus type. The problem was therefore attacked from this angle, and attempts were made to determine how the disease may be transmitted, whether by mechanical means or by insect vector. Experiments were accordingly carried out on transmission by mechanical means and on transmission by insect vector.

EXPERIMENT 1

This experiment was conducted October, 1929, in a five-month-old Smooth Cayenne plantation of the Philippine Packing Corporation in Santa Fe, Bukidnon Province. Three methods known to be employed in transmitting virus diseases by mechanical means were tried. The methods were (*a*) by inserting small pieces of diseased parts into slits made on healthy plants, (*b*) by making incision on healthy plants with a scalpel which had been used previously in chopping diseased plants, and (*c*) by needle pricks as described by Wilbrink(10) for transmitting the sugar-cane mosaic by mechanical means.

Materials and methods.—The first series of transmission was conducted by cutting with a flamed and cooled scalpel a slit at the center near the base of the three middle leaves of ten healthy plants, and inserting in each slit a crushed piece of an infected leaf. For the control similar slits were made on another set of healthy plants, but crushed pieces of a healthy leaf were inserted in them.

The second series of transmission was carried out by making similar slits with a scalpel on the same number of healthy plants

as in the first series, immediately after the scalpel had been used in chopping diseased plants. For the check, similar slits were made on the same number of healthy plants with a flamed and cooled scalpel.

In the third series direct transmission was tried by holding an infected leaf closely against each of the three middle leaves of ten healthy plants and thrusting rapidly ten fair-sized steel needles, mounted at the end of a stick, through the diseased leaf deep enough to prick the healthy leaves. For the control, another set of ten healthy plants was treated similarly but substituting a healthy leaf for the diseased leaf. In every case the surface of the leaves used in the three series was disinfected with 1 : 1,000 mercuric chloride in 70 per cent alcohol and rinsed with sterile distilled water, while the needle was flamed and cooled as in the case of the scalpel.

Results.—Each plant in the experiment was observed daily for a month for signs of the disease, but all except three in the last series remained negative even after six months, indicating that the virus of pineapple yellow-spot is not transmissible mechanically by plant extracts. Johnson and Hoggan(2) place pineapple yellow-spot in the category of the viroses that are transmitted by sucking insects and not mechanically by plant extracts. For this reason the three plants in the third series which showed infection probably were infected before they were used in the experiment.

EXPERIMENT 2

The failure to obtain positive results from the transmission tests by mechanical means suggested that the pineapple yellow-spot virus is transmissible only by an insect vector. Search for this insect vector has revealed the presence of a number of insects such as the pineapple mealy-bugs, springtails, occasionally thrips, etc., both on healthy and diseased pineapple plants. To determine what rôle these insects might play in the transmission of yellow-spot, the following preliminary tests were set out in January, 1930, in a five-month-old Bukidnon Smooth Cayenne plantation of the Philippine Packing Corporation, Santa Fe, Bukidnon Province.

Materials and methods.—Ten plants of uniform size and with typical infection of yellow-spot were collected from the ratoon fields at Diklom and Santa Fe, and brought to field 1. The top portion of each plant was cut and meticulously examined for the insect fauna present which was found to be composed of the

springtails (*Isotoma* sp.), in adult and larval stages; the pineapple mealy bug (*Pseudococcus brevipes*), in different stages; the onion thrips (*Thrips tabaci*), in larval stage only; and *Tarsonemus ananas*.

Table 1 shows that with the exception of the ninth all plants to which the onion trips (*Thrips tabaci* Lindeman) from infected pineapple had been allowed to migrate became diseased. Some of the plants showed the initial symptoms as early as the second week. All other pineapple plants to which either *Isotoma* sp., *Pseudococcus brevipes*, or *Tarsonemus ananas* were allowed to migrate and colonize remained free from yellow-spot infection. The results seem to indicate that *Thrips tabaci* transmitted the pineapple yellow-spot; but the other insects, particularly the springtail (*Isotoma* sp.) which was present on all the specimens, could not transmit the disease. As the virus of pineapple yellow-spot is one that is biologically carried by *Thrips tabaci* and requires an incubation period of about ten days in the body of the insect vector, the writer believes that the thrips, when used, already had the virus in them for some time. The lone negative result from the transmission experiment with *Thrips tabaci* is perhaps due to the fact that the virus has not yet remained in the body of the insect for the required time of incubation, or else the insects were not viruliferous. Kunkel(3) states that *Thrips tabaci* larvæ can obtain the virus of the yellow-spot but the adults cannot.

TABLE 1.—*Preliminary experiments showing positive results from inoculations with Thrips tabaci.*

Plant (immature).	Insects present in tops used.	Inoculated.	Observations; 1930.				
			Jan. 23.	Jan. 30.	Feb. 6.	Feb. 13.	Feb. 20.
1	*Isotoma* sp.;[a] *Pseudococcus brevipes; Thrips tabaci.*	Jan. 16, 1930	−	+	+	+	+
2	----do----	----do----	−	+	+	+	+
3	----do----	----do----	−	−	+	+	+
4	*Isotoma* sp.; *Tarsonemus ananas.*	----do----	−	−	−	−	−
5	----do----	----do----	−	−	−	−	−
6	*Isotoma* sp.; *Pseudococcus brevipes.*	----do----	−	−	−	−	−
7	----do----	----do----	−	−	−	−	−
8	*Isotoma* sp.; *Thrips tabaci.*	----do----	−	+	+	+	+
9	----do----	----do----	−	−	−	−	−
10	----do----	----do----	−	−	+	+	+

[a] Determination by Dr. Fidel del Rosario, entomologist, Bureau of Science.

EXPERIMENT 3

While experiment 2 was still in progress it was observed that many of the weeds, *Emilia javanica* (Burm. f.) C. B. Rob. and *E. sonchifolia* (Linn.) DC., growing about the old plantations, especially around the ratoon fields, were infected by a certain chlorosis characterized by mottling and circular chlorotic blotches with zonate markings which appeared very similar to, if not identical with, the pineapple yellow-spot. Examination of such plants showed the presence of a number of insects, one of which was the onion thrips, *Thrips tabaci* Lindeman. This observation gave rise to the suspicion that the two viroses are probably identical. To elucidate this matter the following tests were carried out February 21, 1930, in Santa Fe, Bukidnon Province.

Materials and methods.—Fifteen uniform Smooth Cayenne crowns, which were obviously free from any kind of spots, were collected from a field where the disease is not known to have occurred. These crowns were trimmed, cured, and dipped fifty minutes in 1.5 per cent soap solution at a temperature of 42° to 43° C. and, after being redried in the sun, each was planted in a gasoline can filled with sterilized soil. The bottoms of the cans were perforated for proper drainage. The cans were then arranged in three groups of five, each group being placed on an insect-proof platform. The platforms were made of bamboos and boards and were provided with cheesecloth covers. To prevent insects from interfering with the experiments, the posts of the platforms were covered with Tanglefoot preparation.

After it has been assured that all of the fifteen plants were free from any disease or pest, three larvæ of *Trips tabaci,* which had previously fed for more than ten days on diseased pineapple plants, were transferred June 1, 1930, to each of the five healthy plants in the first group; while three larvæ of *Thrips tabaci,* which had fed on diseased *Emilia javanica* for the same length of time, were transferred to each of the five healthy plants in the second group, leaving the five healthy plants of the third group free from thrips as check.

The thrips were collected from diseased plants and transferred to healthy plants in the following manner: The rather tight-fitting leaves of the heart or bud of the infected plant were loosened to expose the insects hiding in the spaces between the

leaves. With the help of a camel's hair brush moistened with water, the larvæ of *Thrips tabaci* were picked one by one and dropped into a 50-cc beaker with some water to keep them floating. As soon as the required number was collected the water in the beaker was poured into the bud of the plants on which the thrips were to be colonized so that the insects were transferred to the healthy pineapple with the minimum strain. The same method was followed in the case of the thrips from *Emilia javanica.*

Results.—Weekly observations for the occurrence of the disease on any of the plants in the experiment are shown in Table 2.

TABLE 2.—*Transmission of pineapple yellow-spot virus by Thrips tabaci.*

Plant (immature).	Insect.	Inoculated.	Observations; 1930.				
			June 8.	June 15.	June 22.	June 29.	July 7.
1	Thrips from pineapple	Jan. 16, 1930	−	+	+	+	+
2	do	do	−	+	+	+	+
3	do	do	−	−	+	+	+
4	do	do	−	−	+	+	+
5	do	do	+	+	+	+	+
6	Thrips from *Emilia*	do	−	−	−	+	+
7	do	do	+	+	+	+	+
8	do	do	−	+	+	+	+
9	do	do	−	−	−	−	−
10	do	do	−	−	+	+	+
11	Check (none)	do	−	−	−	−	−
12	do	do	−	−	−	−	−
13	do	do	−	−	−	−	−
14	do	do	−	−	−	−	−
15	do	do	−	−	−	−	−

Confirmation of the results obtained from the first inoculation tests (Table 1) that pineapple yellow-spot is caused by a virus transmitted by *Thrips tabaci* may be seen in Table 2. The same table shows that the virus may be transmitted to healthy pineapple either directly from diseased pineapple or from diseased *Emilia* plants. That the pineapple virosis and the *Emilia* virosis are identical, seems a logical deduction from the fact that, when the thrips used in this experiment were transferred to healthy *Emilia* plants, exactly the same characteristic spottings were reproduced on the leaves. The incubation period ranges

from seven to twenty-five days as shown by the first symptom appearing in each inoculated plant after the introduction of the infective thrips. The infected plants became dwarfed and died without reaching maturity. These results seem to be more or less in accord with those reported by Linford.(4, 5)

As in Table 1, it is also shown in Table 2 that plant 9 did not become infected and has not shown the slightest sign of the disease even after six months. The reason for this was that the thrips had not been allowed sufficient time on the source of inoculum. The results seem to verify the identity of the Philippine yellow-spot of pineapple with the Hawaiian pineapple yellow-spot.

YELLOW-SPOT AND "KAUAI DISEASE"

It has been mentioned in the early part of this paper that the pineapple yellow-spot produces a set of symptoms on the fruits very similar to, if not identical with, the symptoms of the so-called "Kauai disease" originally reported in Kauai Island, Hawaii, by Lyon.(6) To determine, therefore, whether the so-called Kauai disease is an after-effect of the yellow-spot or something else, the following test was made.

EXPERIMENT 4

This experiment was set out July 15, 1930, in field 1 of the Philippine Packing Corporation, Santa Fe, Bukidnon Province.

Materials and methods.—Although it was out of season at the time these tests were started, a sufficient number of plants just beginning to bloom were located in three adjoining blocks. Following exactly the same procedure as that used in experiment 3, the blooming plants were used in transmission work by introducing, to each of the first five, three larvæ of *Thrips tabaci*, which had been feeding on diseased pineapple plants for some time; to each of the next five, three larvæ of the same thrips, which had also fed on diseased *Emilia* plants for some time, leaving the last five uninfested as check. This experiment differs fundamentally from the third, however, in the age of the plants when used in transmission work and in the fact that this one was left in the open, while the third was kept in insect-proof cages.

Results.—Monthly observations were taken until the fruits reached maturity. The results are given in Table 3.

TABLE 3.—*Effect of yellow-spot virus on young fruits.*

Plant (immature).	Insect.	Inoculated.	Observations; 1930.				
			Aug. 15.	Sept. 15.	Oct. 15.	Nov. 15.	Dec. 15.
1	Thrips from pineapple	July 15, 1930	+	+	+	+	+
2	do	do	+	+	+	+	+
3	do	do	−	−	−	−	−
4	do	do	−	−	+	+	+
5	do	do	−	−	+	+	+
6	Thrips from *Emilia*	do	−	+	+	+	+
7	do	do	−	−	+	+	+
8	do	do	−	−	−	−	−
9	do	do	−	−	−	−	−
10	do	do	−	+	+	+	+
11	Check (none)	do	−	−	−	−	−
12	do	do	−	−	−	−	−
13	do	do	−	−	−	−	−
14	do	do	−	−	−	−	−
15	do	do	−	−	−	−	−

It is shown in Table 3 that blooming pineapple plants when infested with infective *Thrips tabaci* do not manifest the presence of the yellow-spot virus as early as the younger plants. It takes about two months for the young fruits, which seem to be the focus of attack, to show any manifestation of the disease, in a somewhat different manner. Some of the fruitlets on one side of the infected fruit became constricted and shrunken, while it is readily seen that the other side continued its growth more or less unhampered, forcing the fruit to bend towards the affected side. Further development of the fruit enhanced the bending as well as the rotting of the affected side, so much so, that in some cases the fruit became greatly deformed and hollow (Plate 4, figs. 3 and 4). This has been observed to be of common occurrence in the field. It is very similar to, if not identical with, the so-called Kauai disease in Hawaii. The plant is otherwise normal.

It is also shown in the same table that, as in the previous transmission experiments, some plants were not infected and remained apparently normal. The failure to transmit the yellow-spot in these cases is attributed to insufficient time allowed the virus to remain in the body of the vector. In some cases it was possibly due to the fact that the insect vectors died before they caused the transfer of the virus.

CONTROL MEASURES

For the present only tentative control measures are recommended. Inasmuch as it has been established that the insect vector lives on weeds like the *Emilia* plants, clean culture is of prime importance. Spraying the infected plants with nicotine-soap solution (0.5 per cent soap and 0.1 per cent nicotine sulphate) to get rid of the thrips may help in minimizing the source of infection and spread of the virus. Dusting with nicotine preparations (tobacco dusts) to keep the thrips away from the young leaves may prove beneficial.

SUMMARY

1. A disease of pineapple, next in importance to mealy-bug wilt and bacterial fruitlet black-rot, is described. It is a virosis known in Hawaii as the pineapple yellow-spot. So far it affects only the Smooth Cayenne variety, in the Philippines.

2. Early infection causes stunting, dwarfing, and the ultimate death of young plants. With mature plants it produces malformation and rotting of some fruitlets on one side of the fruit which may become hollow, as well as the stunting and dwarfing of the crowns. This condition greatly resembles the so-called "Kauai disease" in Hawaii.

3. Evidence has shown that the disease is a virosis transmitted only by the insect vector *Thrips tabaci* Lindeman and not by mechanical means. Transmission may be effected by *Thrips tabaci* from either diseased pineapple or diseased *Emilia* plants, provided the insects had been allowed to feed on the source of inoculum for more than ten days. The incubation period of the virus in the pineapple ranges from seven to twenty-five days.

4. Tentative control measures are given which, if followed, would help the growers to minimize the ravages of the disease.

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ILLUSTRATIONS

PLATE 1

FIG. 1. A young Smooth Cayenne leaf showing early symptom (initial spot) of yellow-spot disease. Note the characteristic circular yellow spot with yellow streak below it. × 1.

2. The same as fig. 1 but far advanced. Note yellow streak below the spot ending in a water-soaked brownish streak at the base of the leaf. × 1.

3. Later stage of the initial spot. Note rotting, chlorosis, and necrosis, with concentric ring or zonate resulting from infection. × 1. (Drawings by the artists, National Museum Division.)

PLATE 2

FIG. 1. Six-month-old Smooth Cayenne grown from a crown, showing early yellow-spot infection. Note stunting and dwarfing of all the leaves. Infection may have set in before the crown was planted. × 1/3.

2. Six-month-old Smooth Cayenne grown from a crown, showing later infection. Note stunting and dwarfing of the central leaves in contrast with the lower apparently normal leaves. This signifies that infection took place at a later date. × 1/3. (Photographs by C. S. Angbengco.)

PLATE 3

As in Plate 2, cut lengthwise to show browned patches in the stem around the attachment of the leaf which had the initial spot. × 1/3. (Photographs by C. S. Angbengco.)

PLATE 4

FIG. 1. A mature Smooth Cayenne fruit showing the effect of yellow-spot infection. Note stunted, shrunken fruitlets ("eyes") at the point of infection. × 0.5.

2. Fig. 1 cut lengthwise to show partly rotten tissues of browned fruitlets. × 0.5.

3. A mature Smooth Cayenne fruit showing the effect of yellow-spot infection in advanced stage. Note rotting fruitlets, forming a hollow. × 0.5.

4. Fig. 3 cut lengthwise to show cavity resulting from rotting tissues of the infected fruitlets. × 0.5. (Drawings by the artists, National Museum Division.)

THREE POISONOUS PHILIPPINE MUSHROOMS

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THREE PLATES

In this paper three poisonous mushrooms are described; namely, *Amanita muscaria* Fr., a deadly poisonous mushroom not heretofore reported in the Philippines; *Lepiota cepaestipes* Fr., a species recently found and tested as poisonous in the Philippines; *Lepiota sulphopenita* Graff, which has been reported previously, but recently found poisonous.

Every year, during the mushroom season, cases of mushroom poisoning are reported in the newspapers, but undoubtedly there have been many other cases that have never been brought to the attention of the public. The number of poisonous species is comparatively small, yet only very few are known or tried by the people. While some may prove poisonous to some persons, they may prove edible to others. Also a particular species of mushroom may be edible in one country, suspected to be poisonous in another, and known to be poisonous elsewhere. It is the purpose of this paper to report and describe not only the edible mushrooms [1] that are newly found in the Philippines, but also those that are known to be poisonous or suspected to be so.

AMANITA MUSCARIA Fr. (Deadly poisonous.) Plate 1.

Amanita muscaria Fr. in Syst. Myc. 1 (1821) 16; Saccardo, Syl. Fung. 5 (1887) 13; Atkinson, Mushrooms (1911) 52–54; Kauffman, Agaricaceae of Michigan 1 (1918) 610–611; Ramsbottom, A Handbook of Larger British Fungi (1923) 25–26; McDougall, Mushrooms (1925) 55; Bresadola, Iconographia Mycologica 1 (1927) 8–9; Swanton, Fungi and How to Know Them (1932) 180.

[1] Mendoza, J. M., and S. Leus-Palo, Philip. Journ. Sci. 53 (1934) 223–227.

Pileus [2] at first ovate to nearly hemispherical, when expanded convex, becoming flat in age, 8 to 15 centimeters broad; margin striated at maturity; surface viscid and moist when young, yellow, sometimes orange, rarely whitish, covered with numerous scattered rather compact whitish or pale yellowish scales or warts. Gills free, crowded, white to yellowish, broadest toward the margin. Stipe cylindrical or tapering, bulbous at the base, stuffed when young, becoming hollow in age, white or with yellow tinge, covered with loose and floccose scales. Annulus white and very fragile. Volva much torn in the form of concentric scales or interrupted rings surrounding the lower part of the stem and the bulbous base. Spores subglobose or broadly oval, hyaline, smooth-walled, 8.10 to 9.60 μ long, 6.30 to 7.50 μ broad; average 8.70 μ long, 6.70 μ broad. The flesh is white, tasteless, and odorless. This mushroom grows on the ground, singly or scattered in small groups.

LUZON, Mountain Province, Baguio, *Phil. Nat. Herb. 2161 W. H. Brown,* June, 1933, on the ground; *Bur. Sci. 55683, 55778 W. H. Brown,* June 12, 1933, on the ground.

Examined specimens were compared with a United States collection of C. L. Shear, Bureau of Plant Industry, pathological and mycological collections, No. 66879.

This mushroom is deadly poisonous. It grows in the Philippines in cool moist places in high altitudes. It is quite common in Baguio, Mountain Province and vicinity. In the United States and some European countries the infusion of this mushroom is used as fly poison, hence it is commonly called fly agaric in those countries.

LEPIOTA CEPAESTIPES Fr. (Poisonous.) Plate 2.

Lepiota cepaestipes FR., Epicrisis (1836) 38; SACCARDO, Syl. Fung. 5 (1887) 43; KAUFFMAN, Agaricaceae of Michigan 1 (1918) 640; RAMSBOTTOM, Handbook of the Larger British Fungi (1923) 30.

Pileous 3 to 9 centimeters broad, white or yellowish, mealy or covered with yellowish, floccose or wartlike scales, umbonate, oval then campanulate-expanded, obtuse, soft, striate on the margin and splitting in age. Stem white, pale brown in the early part or late in the mushroom season, otherwise the same color as the cap, base bulbous, somewhat ventricose, tapering upward and occasionally covered with floccose particles, hollow,

[2] The following descriptions are based on fresh full-grown specimens collected during the mushroom season.

5 to 14 centimeters long, 5 to 8 millimeters thick at the apex. Annulus narrow, thin, membranaceous, subpersistent. Gills free, thin, white, narrow, at length remote, edge pruinose. Spores elliptic to ovate, colorless, granular, guttulate, smooth-walled, varying in size from 8.40 to 12.0 μ long, 6.0 to 9.0 μ broad; average 10.0 μ long, 7.0 μ broad. Basidia clavate, 29.0 to 46.0 μ long, 11.4 to 13.5 μ broad; average 30.7 μ long, 12.7 μ broad.

Cæspitose. On decayed leaves under trees and very common during the wet season, from May or April, sometimes up to February when the rainy season is long.

LUZON, Tayabas Province, Atimonan, *Bur. Sci. 55105, 55144 J. M. Mendoza,* June 22, 1933, on the ground: Manila, *Phil. Nat. Herb. 919 Jesus Conosa,* January 15, 1934, on the ground; *Bur. Sci. 50495 S. Leus,* June 21, 1928, on the ground; *Bur. Sci. 50496 B. Reyes,* August 19, 1928, on the ground; *Bur. Sci. 55056 J. M. Mendoza,* July 8, 1933, on the ground; *Bur. Sci. 55117 J. M. Mendoza,* August 1, 1933, on the ground; *Bur. Sci. 55121 J. M. Mendoza,* July 22, 1933, on the ground; *Bur. Sci. 55122, 55123, 55127 P. S. Gener,* September 20, 1933, on the ground; *Bur. Sci. 55135, 55142 J. M. Mendoza,* July 7, 1933, on the ground; *Bur. Sci. 55147 J. M. Mendoza,* June 29, 1933, on the ground; *Bur. Sci. 55148 J. M. Mendoza,* July 11, 1933, on the ground; *Bur. Sci. 55157, 55158, 55161 J. M. Mendoza,* July 11, 1933, on the ground; *Bur. Sci. 55207 J. M. Mendoza,* July 26, 1933, on the ground; *Bur. Sci. 55296 D. Farol,* June 3, 1933, on the ground; *Bur. Sci. 55302 J. M. Mendoza,* June 3, 1933, on the ground; *Bur. Sci. 55611 J. M. Mendoza,* June 19, 1933, on the ground; *Bur. Sci. 55770 P. S. Gener,* November 8, 1933, on the ground; *Bur. Sci. 55771 P. S. Gener,* November 7, 1933, on the ground; *Bur. Sci. 55824 P. S. Gener,* October 13, 1934, on the ground; Rizal Province, Pasay, *Bur. Sci. 55553 J. M. Mendoza,* May 20, 1933, on the ground; *Bur. Sci. 55146 J. M. Mendoza,* July 7, 1933, on the ground; *Bur. Sci. 55151 J. M. Mendoza,* July 29, 1933, on the ground.

This mushroom is one of the most resistant to drought. During the mushroom season it is one of the first and last to be seen appearing in shady places, especially under acacia [*Samanea saman* (Jacq.) Merr.] trees where semidecayed leaves and other organic matter are thickly piled in heaps. It often grows in large quantities, forming a large mat. The features of this fungus are very variable, especially the size and color shading. In the early and late parts of the rainy season this mushroom is

much smaller than the normal size. The stem is chestnut on the lower portion, becoming paler to almost white toward the gills. The stem at this time is slightly winding and the texture is hard. The pileus is also pale chestnut at the upper central portion, becoming much paler to white toward the margin. It is thinner and much narrower than the average-sized cap.

This mushroom is poisonous, specially when a large quantity is eaten. Tests have been made as to its edibility. For the first time four pieces of the average size were cooked together with a very little lard, pieces of garlic, onion, ginger, and dried salted fish (*tinapá*). The prepared dish was eaten by the author together with the regular noon meal in the laboratory. The taste was excellent and the same as that of *Lepiota americana*,[3] which is delicious and a little peppery. Encouraged by the result of the first test and because of the delicacy of the dish, the senior author took home about a dozen mushrooms of this species and cooked them in the same way as before. Four persons, besides the author and his family, ate the mushroom dish together with other food for supper. It was delicious as before and nothing happened. The next day a matlike form of this mushroom was found growing under the acacia trees in the ground of the Bureau of Science. These were collected and a regular-sized paper bag full of them was taken home for supper. The cook cleaned the mushrooms at once and washed them well with water. They were torn into pieces of the desired size and cooked as before. A small quantity of the soup of salted fish (*bagong*) was also added later when the mushrooms were almost cooked. Four persons, besides the senior author, and two members of the family partook of the mushroom dish. The portions, however, taken by them were not the same in amount. The cook, his wife, and a maid took the largest ration, while the rest of us took but very little of the dish. About half an hour after supper, three of the persons who ate the biggest portion, began complaining of slight pain in the stomach, accompanied by dizziness. Soon they began vomiting continuously, until every bit of the mushroom was completely discharged from the stomach. No diarrhœa was observed among the victims. Nothing happened to the fourth person who ate a less amount, nor to the rest of us who took but very little

[3] Mendoza, J. M., and S. Leus-Palo, Philip. Journ. Sci. 52 (1934) 223–227.

of the mushroom dish. The victims became weak and perspired profusely. When vomiting was over, they lay down quietly and went to sleep. A doctor was consulted, and an emetic was prescribed. The medicine was no longer taken, for it was thought to be unnecessary.

The incident proved beyond doubt that *Lepiota cepaestipes* is poisonous, especially when a large amount is eaten. When taken in small quantities, however, no poisonous effects were observed.

This mushroom is known to be edible in the United States, while in England [4] it is suspected of being poisonous. The same is true of some other species of mushroom; for example, *Volvaria speciosa* is considered poisonous in Europe, whereas in California [5] it is eaten and considered perfectly safe.

An interesting habit of this mushroom is the formation of a fairy ring about 2 meters in diameter. The ring was found on green grass, in Atimonan, Tayabas, in June, 1933. This was the first incidence of the formation of a fairy ring by this particular mushroom ever reported in the Philippines or elsewhere.

LEPIOTA SULPHOPENITA Graff. (Poisonous.) Plate 3.

Lepiota sulphopenita GRAFF, Philip. Journ. Sci. 9 (1914) 245.

Pileus globose to conical when young, becoming campanulate to broadly expanded, generally sulphur yellow throughout, sometimes the center of the cap is nearly orange, covered with fine floccose scales which disappear in age, thin, fleshy, 4 to 7 centimeters broad; margin deeply striated, specially when old. Gills free, thin, narrow, few, white, becoming yellowish because of the scales from the cap that adhere to them. Stem hollow, floccose, cylindrical, bulbous and elongated at the base, sulphur yellow, 7 to 10 centimeters long, 6 to 11 millimeters broad. Annulus thin, and early disappearing. Spores ovate-elliptic, colorless, guttulate, smooth-walled, 7.5 to 9.6 μ long, 5.4 to 6.3 μ broad. Basidia clavate, granular, 15 to 18.6 μ long, 3.6 to 4.5 μ broad.

The flesh is yellow. The plants grow solitary or gathered in tufts of a few.

LUZON, Manila, *Phil. Nat. Herb. 1283 P. S. Gener*, May 28, 1934, on the ground; *Phil. Nat. Herb. 1345 P. S. Gener*, May

[4] Ramsbottom, John, A Handbook of the Larger British Fungi (1923) 30.

[5] McDougall, Mushrooms (1925) 62.

23, 1934, on the ground; *Merrill 8417* (type in the Phil. Nat. Herb.), August 18, 1912; *Bur. Sci. 55825 J. M. Mendoza,* August 31, 1933, on the ground; *Bur. Sci. 55826 P. S. Gener,* October 8, 1933, on the ground: Rizal Province, Pasay, *Phil. Nat. Herb. 1547 Bureau of Health employee,* May 23, 1934, on the ground.

A batch of this mushroom was sent by the Bureau of Health to the Bureau of Science for determination. According to the investigation conducted by the Bureau of Health officer, this mushroom was the cause of poisoning of four persons in Pasay, May, 1934; one of them, a child, died.

ILLUSTRATIONS

Plate 1

Fig. 1. *Amanita muscaria* Fr., × 0.6.
2. *Amanita muscaria* Fr., a section of the gill; photomicrograph showing the hymenium with basidia and spores; × 235.

Plate 2

Fig. 1. *Lepiota cepaestipes* Fr., in natural habitat, showing different stages.
2. *Lepiota cepaestipes* Fr., a section of the gill; photomicrograph showing the hymenium with basidia and spores; × 235.
3. *Lepiota cepaestipes* Fr., forming a fairy ring.

Plate 3

Fig. 1. *Lepiota sulphopenita* Graff, showing various stages.
2. *Lepiota sulphopenita* Graff, a photomicrograph of the spores from spore collection, × 433.

1

2

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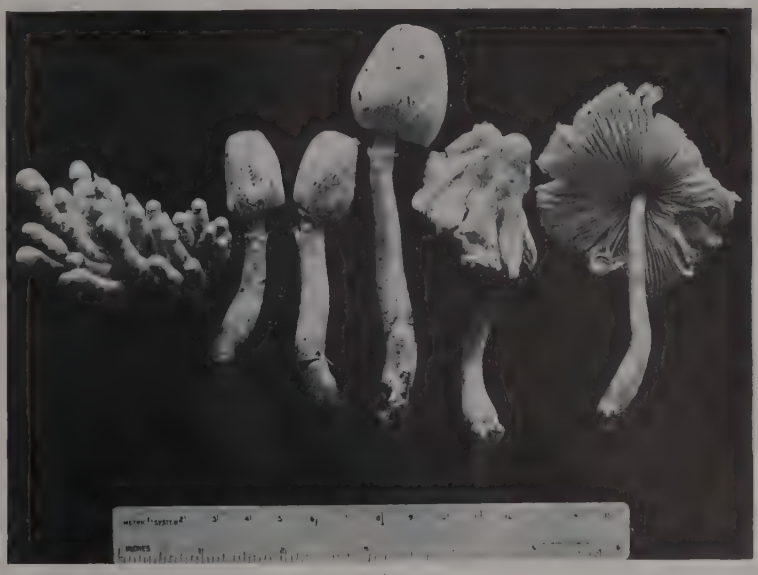
PLATE 2.



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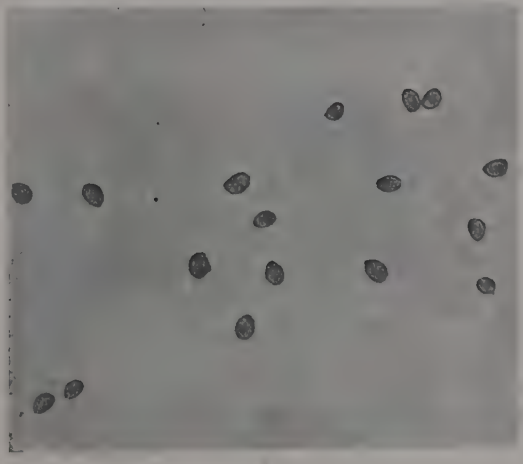

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PLATE 3.

ETHNOGRAPHIC NOTES ON THE ISINAIS OF NUEVA VIZCAYA

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THREE PLATES

Of the minor Christian groups in the Philippines, the Isinais, or Ituyes, are one of the least known. They are made up of converts from the lowland group of people of uncertain origin having the same characteristics as the Pangasinanes, and intermarried with the Igorots.

ORIGIN

The name Isinai was given to this group by the Italones, Ilongots, or Ibilaos, who occupy the headwaters of Cagayan River in Nueva Vizcaya Province, Luzon. Pardo de Tavera says that the etymology of the word *Isinai* seems not very clear. *Sina,* in the Iloko dialect means "segregation, or separation, of persons;" the word seems acceptable as root of the name of the tribe, when one considers, for example, that the name Ilongot is formed from the word *longot,* which in the Iloko dialect means "reunion, or aggregation, of persons."

Su etimología no me parece muy clara; *sina,* en ilocano significa "segregación, separación de personas", voz que me parece aceptable como radical de este nombre de tribu, cuando se considera que otra de ellas llamada *ilongot* está formada de la palabra *longot,* que en ilocano quiere decir "reunión, conjunto, agregación de personas."[1]

GEOGRAPHIC DISTRIBUTION

At present the Isinais are confined to the three mountain towns of Aritao, Bambang, and Dupax in Nueva Vizcaya Province, north-central Luzon. These three towns were established about the middle of the eighteenth century. Aritao was established in 1740 with 984 inhabitants, Bambang in 1747 with 3,639 inhabitants, and Dupax in 1740 with 2,489 inhabitants.

[1] Pardo de Tavera, T. H., Etimologia de los Nombres de Razas de Filipinas. Manila (1901) 13.

ORIGIN OF THE NAME OF EACH TOWN

Aritao derives from the word *hari-tao*, meaning "our king." It is said that there was a strong *Igolot* (Igorot) living on the top of Bona Mountain near Aritao. He was persuaded by the Spanish missionaries to come down to the lowland so that he could be Christianized. He finally consented on condition that the place he selected for his home in the lowland be filled with earth from the top of the mountain where he was living and that his house be transferred to the lowland. As he was very strong the people of the lowland were afraid of him. They call him "Aritao."

Bambang means "a hole dug in the ground." Formerly there were two clans in the town of Bambang, who were constantly at war against each other. At last they realized the great number of casualties in both clans and came to an agreement to settle their differences. They buried their weapons in a big hole (bambang) as a sign of peace.

Dupax derives from the Isinai word *dupa*, meaning "to rest or recline." The town was formerly a famous hunting place for deer. There were two native chiefs, who went to hunt deer in that place; after hunting, they use to take a rest there.

POPULATION

Once the Isinais were numerous and occupied several areas in Nueva Vizcaya Province. Now they are fast disappearing due to intermarriage with Ilocano settlers, particularly those living in the town of Aritao and Bambang. In Dupax, however, there are still many pure Isinais. Their estimated number is over 2,000.

PHYSICAL TYPE

The Isinais are predominantly of the Malay blend, with a very high percentage of the short Mongol type. There is probably also some Ainu admixture.[2]

CULTURE

Formerly the Isinai culture showed some relationship with that of the Ifugaos and of the Ibaloi group of the Igorots.[3] Now much of the general Spanish-Filipino culture prevails.

[2] Beyer, H. Otley, Population of the Philippine Islands in 1916. Manila (1917) 48.

[3] Beyer, H. Otley, op. cit. (1917) 48–49.

ECONOMIC LIFE

Agriculture.—The Isinais practice irrigated agriculture. Their principal products are rice, cotton, tobacco, and coconuts.

Hunting and fishing.—Hunting and fishing are carried on to some extent. Nets and traps are employed for catching fish. The fish traps are unique. Those used for catching murrels are called *bangcat* and are provided with rattan spines inside. Those used for catching small fish are called *apayao*.

Basketry.—The Isinais do some basket work. The materials commonly used are rattan and nito. Two types of weave are employed; namely, twilling, or sawali weave, and coiling. The traveling baskets, which are called *pasiquing*, are similar to those of the Ilocanos, and the Ifugaos and other pagan groups of northern Luzon.

Weaving.—The Isinais do excellent weaving. They employ "the tie and dye process" on warp similar to that of the Mandayas and Bagobos of Mindanao. The designs are unique and characteristic. They are usually the highly conventionalized forms of human figures, animals, and other objects. These designs are seen particularly on their *ues pinutuan* (blankets) and *indong* (G strings). Stripes of red and yellow or red and black are employed on their *caray* (traveling bags).

Transportation.—The Isinai methods of transportation are primitive. Sleds and carts drawn by carabaos or cattle are used to transport products from one place to another. Horses are also in common use on mountain trails.

Housing.—The Isinai house is a structure of wood with a thatched roof. The floor is raised from four to six feet above the ground. The date of the construction of the house is usually carved on the ridgepole. The piled stone fence around the house, which is about one meter high in the form of a trench, is common throughout the three Isinai towns. The stones are obtained from shallow streams nearby.

Dress.—The typical hat of the Isinais is the *taddung* or a round hat made of *dap-dap* wood (*Erythrina variegata*). Their dress is similar to that of other Christian Filipinos. The ordinary clothing of the men is the shirt and trousers; the women wear *camisa* and skirt.

SOCIAL LIFE

Dances.—The typical dances of the Isinais, which are held on festive occasions, are four; namely *investida, induma, in España,*

or *itaquero*, and *aninó*. Each of these dances requires a man and a woman. In the investida the dancers do not hold each other, and the movements of the hands follow the rhythm of a particular music. In the induma the movements of the feet emphasize the rhythm of a particular music. In the in España, or itaquero, the dancers one after the other recite some melancholy verses as a sort of poetical joust. The aninó is danced to the accompaniment of a guitar. The dancers recite some verses, which are a sort of advice to the newly married couple.

Marriage customs.—Formerly it was customary for the parents to make the arrangements for the marriage of their children; now this matter is left to the boy and the girl. When a boy wants to marry, his parents inform the parents of the girl that they will go to the latter's house at a stated date and hour. This is called *balita*.

Then the parents of the boy, together with a spokesman, inform the girl's parents that the boy and the girl love each other. They provide an artificial heart, which is called *calun* and contains a ring. The boy gives the ring to the girl as a sign of troth. A cup of native wine is also provided, one-half of which is taken by the boy and the other half by the girl. After drinking, both announce before their relatives and friends that they are lucky enough to be future husband and wife.

Then comes the marriage ceremony, which is usually performed in a catholic, a protestant, or an Aglipayan church.

After the wedding, the couple goes to the girl's house where they entertain their relatives and friends. Just after lunch or dinner the newly married couple dances the investida. The relatives and friends of both parties likewise dance and after dancing put their money on the plate for the couple. This is called *paragala*.

The boy does not live or sleep with his wife just after their wedding. On the next day his parents turns him over to the parents of the girl. Only then can they live together as husband and wife.

Circumcision.—Like other Christian Filipinos, the Isinais practice circumcision. They split the foreskin with a sharp knife or razor. Young guava leaves are masticated and applied as a medicine. The boys are usually circumcised at the age of 14 to 16. They call this practice *capun*.

Superstitious beliefs.—The Isinais believe that sickness is caused by the *anitos*. They offer pigs and chickens to the anitos

in order to appease them. The person who finds out the causes of sickness is called *sipu.*

When one loses his way in the forest, it is believed that it is caused by the anitos. The person who loses his way is called *naib-iba.*

Death.—When a member of the family dies, his belongings are usually placed in one corner of the house, and ashes are put on the stairs in order to detect if the spirit of the dead has visited them. If they want to see the shadow of the dead, they place a white curtain in the house. The name of the person and the date of his death are usually written on the front wall of the house. As a sign of mourning the women wear black ribbons.

DIALECT

Of the many minor dialects spoken in the Philippines, which are rapidly becoming extinct due to the encroachment of their strong neighbors, Isinai, or Inmeas, is one of the least known.[4] It is spoken only in the three mountain towns of Aritao, Bambang, and Dupax, Nueva Vizcaya Province. The dialect, although distinct, is related to Iloko.

The Isinai postpositive article.—Conant [5] states that the most striking characteristic of the Isinai dialect is its treatment of the definite article. Its forms are the following: *Ar, ardari,* and *war.* Of these forms, *ar,* is both prepositive and postpositive, *ardari* positive only, and *war* prepositive only. All words requiring the definite article take a postpositive, *ar* for the singular and *ardari* for the plural; for example, *tahu,* man; *tahuar,* the man; *tahuardari,* the men. A prepositive, *ar* or *war,* accompanies the postpositive under the following syntatic conditions: *War* is used when the word stands in the relation of subject of a copulative verb, and *ar* when it stands in the relation of predicate. The following example will illustrate both cases: *War Espiritu Santoar ot ar atlunar personas;* The Holy Ghost is the third person. *Ar* and *war* are used for singular and plural alike; for example, *ar* (or *war*) *babayardari,* the women; but they must always be accompanied by the postpositive article. Under all other syntatic conditions, the postpositive article only is used; for example, *namalsat biopar on pio'ar*—made the heaven and earth.

[4] Conant, Everett Carlos, Journ. Am. Oriental Soc. 35 (1915) 289.
[5] Journ. Am. Oriental Soc. 35 (1915) 291.

Lexical comparison.—Some of the commonest terms have been selected to show the affinity of the Isinai dialect to its sister tongue, the Iloko, and to gain thereby an insight into the nature of the features common to both dialects.

Common terms showing the affinity of the Isinai to the Iloko.

English.	Isinai.	Iloko.
moon	buen	bulan
night	labi	rabi-i
star	bituon	bittuen
lightning	ilat	quimat
fire	apoy	apuy
water	danum	danum
year	taon	tauén
flower	tabong	sabong
root	lamot	ramut
vein	ulat	urat
man	tajo	taó
father	[illegible]	amá
woman	babai	babai
mother	ina	ina
old man	lacay	lacáy
[illegible]	ñgaron	nágan
head	uyo	ulo
lip	subil	bibig
tongue	dila	dila
eye	mata	mata
breast of female	tutu	susú
armpit	quere	quili-quili
heart	pusu	púso
leg	ipuo	luppo
nail	uu	cocó
finger	am-ama	ramai
elbow	sico	sico
gall	apdo	apró
dog	atu	ásu
fish	isira	sidá
pig	baboy	babuy
goat	canding	calding
deer	[illegible]	ugsa
house	beyoy	balai
food	[illegible]	canen
fat	taba	tabá
gold	balitu	balitoc
hunger	bisil	bisin
thirsty	naoao	uáo
[illegible]	csa	maisa
two	dúa	dua

Common terms showing the affinity of the Isinai to the Iloko—Continued

three	tio	tal-ló
four	upat	up-pát
five	lima	lima
six	unum	inném
seven	pitu	pito
eight	ueyo	oalo
nine	siyam	siám
ten	simpio	sangapulo
eleven	simpio ta osa	sangapulo ket maisa
twenty	duampio	duapulo
one hundred	osan lasut	sangagasut

ILLUSTRATIONS

Plate 1

Fig. 1. An Isinai house (beyoy). Note the piled stone fence about 1 meter high in front.
2. A typical saddle of the Isinais made of rattan.

Plate 2

Fig. 1. A fish trap (bangcat) for murrel fish provided with rattan spines inside.
2. A pot lifter made of rattan.
3. A typical blanket (ues pinutuan) of the Isinais woven into "tie and dye" process with realistic designs.

Plate 3

Typical Isinai G strings (indong).

1

2

PLATE 1.

1

2

3

PLATE 2.

PLATE 3.

SOME METHODS OF CIRCUMCISION IN THE PHILIPPINES

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TWO PLATES

INTRODUCTION

"Circumcision" in Latin is *circumcisio,* derived from the Latin verb *circumcidere—circum* (around) plus *caedere* (to cut), meaning "to cut around." It is locally known as *tuli* among the Tagalogs; *turi* or *tulé* among the Visayans; *tulí* among the Pampangos; *coguit* among the Ilocanos; *turi* among the Bikols; *segat* or *capon* among the Pangasinanes; *tuli* among the Sambals; *banguit* among the Yogads; *sugiat* among the Kalingas; and *islam* among the Sulu Moros. It is defined in the Encyclopædia Britannica as the removal of a sufficient portion of the foreskin to allow a free retraction beyond the glans penis. In the Americana it is defined as an amputation of the foreskin or labia minora of the human organs of generation. From the methods practiced in the Philippines there is but one that conforms to the above definition, known as *tuli sa bao.* All other methods may be defined as the lengthwise slitting of the foreskin, or the prepuce of the penis, so as to expose the head of the penis as far as a little behind the corona glandis. The method now employed by many in the Philippines is circumcision in the real sense performed by physicians. It is the removal, or the amputation, of the foreskin of the penis and the suturing at the free borders of the prepuce under local anæsthesia.

HISTORICAL BACKGROUND

Circumcision is a very generalized ancient custom, and few Filipinos have not undergone the operation. It is an old practice, for the natives have acquired it from many generations before them.

> Some of them, who practiced circumcision, traced its origin not to the Mohametans (inhabitants of Borneo 300 leagues distant) with whom they had no acquaintance, but to their own very remote ancestors.[1]

[1] Blair, Emma Helen, and James Alexander Robertson. The Philippine Islands 1493–1898 **34** (1905) 319.

However, it must not be inferred that this custom has been acquired from the Jews or from the Arabs.

> They had the custom of circumcision, a custom which they did not acquire from the Arabs, since it is still practiced on the peaks of the independent mountains. They practice it still, and that against the will of the curas. Ancient customs have very great force. It is to be noted that the manner of operation is not the same as that practiced by the Jews, for the cut is made from the upper to the lower part.[2]

It is interesting to note also that female circumcision had been in practice together with male circumcision, but due perhaps to the unappreciated advantages that it gives it did not gain ground and had been forgotten.

> Not only do they circumcise the males but also the women, girls, or *dalagas* [an operation] which they call *sonad*. It is reduced to cutting the organ or opening it up somewhat.[3]
>
> At the birth of males and even the females, the midwives themselves made easier for them the carnal act, by cutting off I know not what from their organs.[4]

METHODS USED

So far there are four generally known methods used; namely, *tuli sa batakan*, *tuli sa itak*, *tuli sa gunting*, and *tuli sa bao*. These methods are performed by any man whom the one to be circumcised may request. Preferably, a barber is chosen because of his skill in handling the cutting instruments used, but never a relative of the one to be circumcised is chosen. This operation is done in a very secluded place during the hot season. The boy to be circumcised must be at the age ranging from 8 to 14 depending upon the preputial adhesion. A bath before the operation is required in order to make the cutting of the skin easy.

Tuli sa batakan.—The operator provides a polished piece of wood preferably a guava tree branch, carved and flattened at one end. The length is about 1½ ft. One end of it is driven into the ground at the same elevation as that of the penis when the one to be operated squats on his thighs. The foreskin or the prepuce of the penis is then drawn to the flat point of the erected apparatus called the *batakan*. The operator also in a squatting position, picks up the knife with one hand and places it lengthwise on the surface of the drawn foreskin. With a

[2] Op. cit. 40 (1905) 220.
[3] Op. cit. 43 (1905) 110.
[4] Op. cit. 40 (1905) 365.

stick on the other hand he gives a sharp blow. If he does not succeed in the first blow he gives the second until he exposes the head of the penis. In some instances, the operated person faints, and in this case the operator slaps his face to bring back consciousness, then applies the medicine and dresses the wound and the boy is considered baptized (*biñagan*) (Plate 1, fig. 1).

Tuli sa itak.—A well-sharpened bolo (*itak*) is provided for this operation. The point of the bolo is inserted lengthwise between the prepuce and the head of the penis while the handle drops to the ground. Both the operator and the one to be operated are in a squatting position. With the left hand of the operator drawing the prepuce on the edge of the bolo towards him, he strikes the prepuce with a piece of banana leaf petiole in his right hand until he succeeds in exposing the head of the penis. Medicine is applied, the wound dressed, and the operation is finished (Plate 1, fig. 2).

Tuli sa gunting.—A pair of sharp scissors (*gunting*) is necessary for this method. One point of the scissors is inserted through the opening of the prepuce. Then the prepuce is stretched lengthwise on the edge with one hand and with the other slitting is accomplished. The wound is treated, dressed, and the process is over (Plate 2, fig. 1).

Tuli sa bao.—This method is an imitation of the Chinese way of circumcising their boys. It is commonly known as *tuling intsik.* Of all the mutilations described above this is the most sacrificial one because above all, the wound is circular in nature and besides the time needed in healing is quite long, for the cut borders of the prepuce could hardly be kept together as they are not sutured. The instruments needed are a polished coconut shell (*bao*) with a hole in the center and a razor. The prepuce is inserted through the hole and is stretched forward so that the head of the penis rests behind the hole of the shell. The victim holds the shell firmly with his two hands, while the circumciser with his hand holding the stretched prepuce amputates it crosswise. The wound is treated and dressed and the person is considered circumcised (Plate 2, fig. 2).

The medicine applied in all of the operations described is young guava leaves masticated in the form of a paste either in the mouth of the operated or the operator. The dressing material used is a circular or rectangular piece of cloth with a hole in the center, termed *baro* locally. The size of the

hole is determined by the diameter of the penis. The head of the penis passes through the hole and the baro is fastened against the corpus penis so as to keep the cut borders of the prepuce together. A day after the operation the wound is thoroughly washed with a solution made by boiling guava leaves in water. This process of cleansing the wound is locally known as *langas*. After this fine scrapings of coconut shell are applied. Then the penis is held over the smoke produced by burning fresh jackfruit leaves, a procedure calle *suob*. This is done so as to reduce the swelling of the penis. Once a day the wound is washed and dressed with new materials. This process continues until the wound is perfectly healed. The usual treatment lasts from ten days to one month.

Aside from the artificial methods of circumcision there is what is called natural circumcision. The prepuce by nature is very short and even if the foreskin is not mutilated the head of the penis comes out naturally after the preputial adhesion has been destroyed. In such a case circumcision is no longer needed for this is already in a prophylactic condition.

WHY EVERY MALE PERSON SHOULD BE CIRCUMCISED

Circumcision does not stand alone as a bodily mutilation for beauty. Unlike other mutilations, such as those performed on the skull, face, teeth, skin, finger, and toe which are merely done by mankind to secure permanent amulet or charm, circumcision arose from a protective motive.

To speak of circumcision folklore may seem to imply an irrevent view of a practice which has acquired great theologic, ethnic and prophylactic significance.[5]

Circumcision has been advised in most cases for one of three reasons: First, a long redundant foreskin, with or without preputial adhesions. Second, a varying degree of irritation about the genitalia, especially the foreskin. Third, a varying degree of balanitis (inflammation of the glans penis).

Other less common reasons assigned as an indication for circumcision are: enuresis, masturbation and nervousness.[6]

The operation is performed chiefly for purposes of cleanliness and to facilitate removal of the smegma, which normally collects beneath the foreskin, but sometimes is necessary to allow free passage of urine. For this purpose it is done as a preventive measure in the infant. In adults the

[5] Kierman, James G., Urologic and Cut. Rev. 25 (1921) 608.
[6] Lanman, Thomas H., Boston Med. & Surg. Journ. 190 (1924) 628.

chief reason for its performance is balanitis or inflammation beneath the foreskin due to lack of cleanliness or to the presence of a venereal sore; in this case circumcision is a necessary part of the treatment.[7]

. . . all), however, circumcise themselves, saying that they do it for their health and for cleanliness.[8]

This operation has been in practice for a long time, and nearly all have submitted themselves to it. An uncircumcised person is considered ridiculous and is taunted by his friends by the terms *suput, suputino, apog, sungao,* and *buenos días.*

Among them being uncircumcised is looked upon as a defect so much so that children of both sexes cruelly taunt those who have reached the age of puberty and are still uncircumcised. They apply to them with the intent to insult the term *suput,* which originally meant "constricted" or "tight", but has come to mean "one who cannot easily gain entrance in sexual intercourse." When American troops first came to Hagonoy and bathed in the river, the fact that they were uncircumcised was a subject of great gossip in the market place.[9]

[7] Encyclopœdia Britannica 5 (1929) 720.
[8] Blair and Robertson, op. cit. 5 (1905) 119.
[9] Barney, Charles Norton, Journ. Assoc. Milit. Surgeons U. S. 13 (1903) 159.

ILLUSTRATIONS

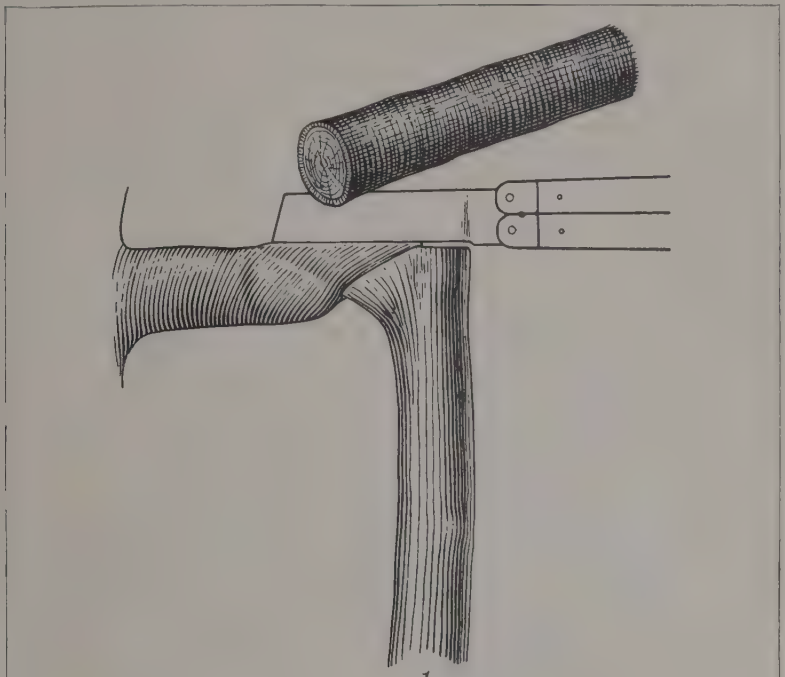
[Drawn by V. V. Marasigan, H. T. Costelo, and R. C. Aguilar, of the National Museum Division.]

PLATE 1

FIG. 1. The *tuli sa batakan.*
2. The *tuli sa itak.*

PLATE 2

FIG. 1. The *tuli sa gunting.*
2. The *tuli sa bao.*
3. Penis before circumcision.
4. Penis after slitting, showing the length of the slit.
5. A circumcised penis.



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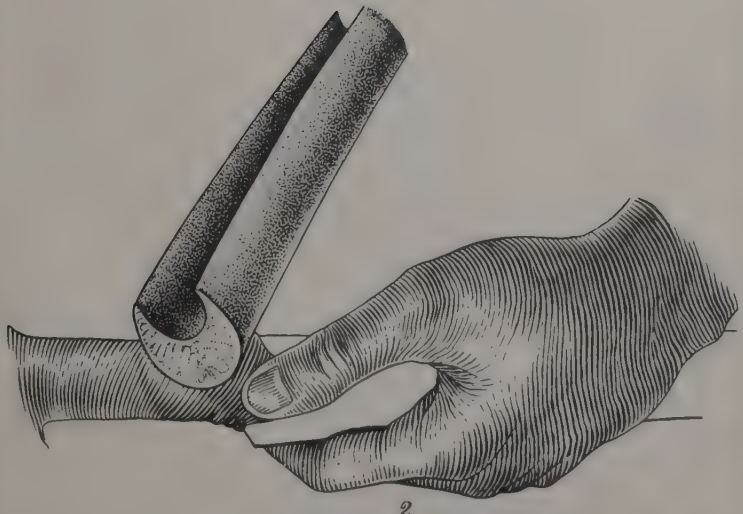

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PLATE 1.

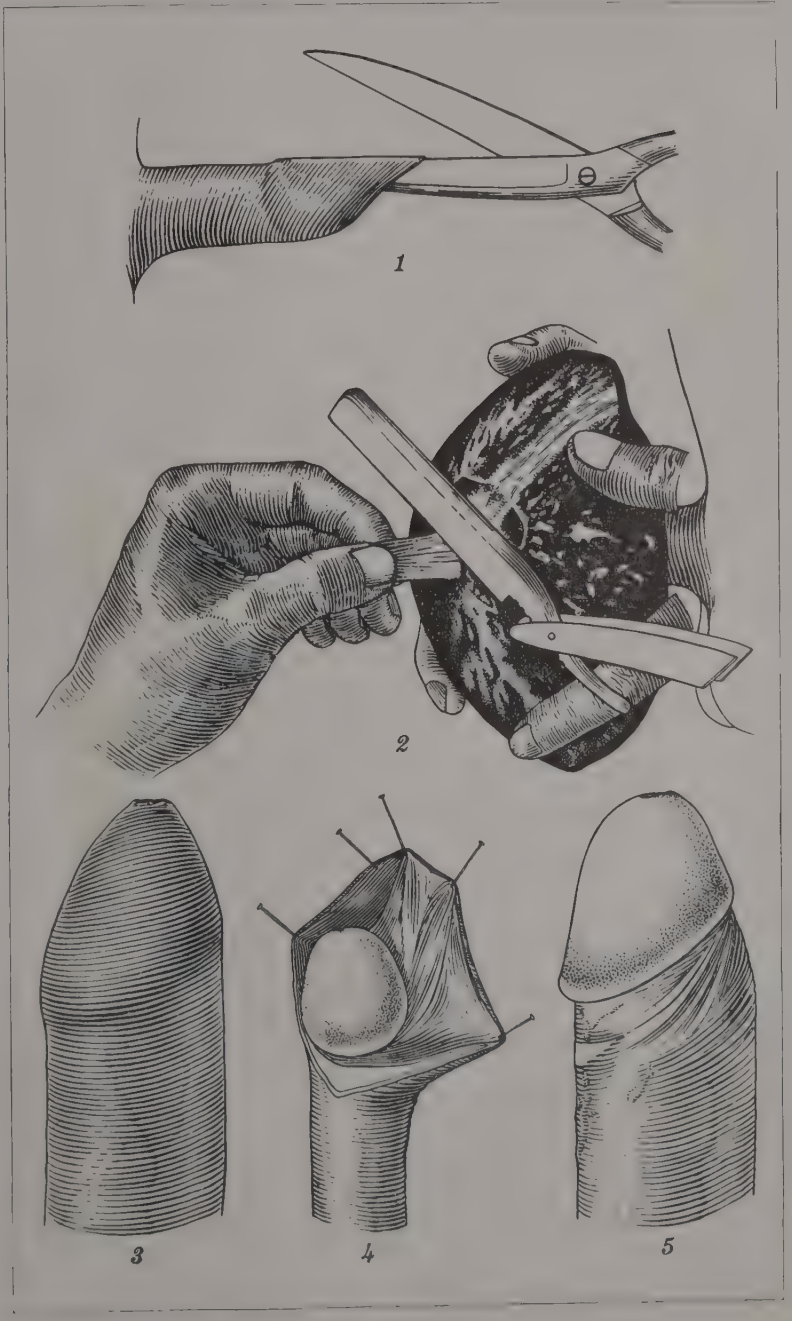

PLATE 2.

THE CULTURE OF THE SIAMESE FIGHTING FISH BETTA SPLENDENS [1]

By José S. Domantay

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Betta splendens Regan, commonly known as the Siamese fighting fish, because of the instinct of the males to fight when placed together, was introduced into the Philippines from Siam about four or five years ago. During its first year in the Philippines it was the most expensive ornamental fish. A pair commanded as much as 40 pesos. Later, the price fell to 25, to 20, to 14, and so on, to as low as 1 peso at present.

January 2, 1934, a pair of *Betta splendens* was given to the writer by Mr. Lek Laksanahuta, a Siamese. According to the information given by Mr. Laksanahuta, this fish is very common in Siam and is found even in places where carabaos wallow, in small ponds, and rice fields. In Siam the male is used in place of the game cock for purposes of betting.

Breeding.—Before starting to breed, one must decide on the color or colors of the fish desired. Likewise, he must know the mode of transmission of the different colors if he is to control the kind to be produced. Although there are many kinds of fighting fishes of different colors now available in the market, there are only two principal groups. One of these is the Cambodian fighting fish, whose body color is typically creamy white and fins and tail are reddish. The other is the Siamese fighting fish, whose body and fins are either greenish blue, gray, reddish, or violet. According to the preliminary breeding results of Dr. L. S. Clemente, of the Department of Zoölogy, University of the Philippines, who is doing research along this line, the color of the Siamese fighting fish, irrespective of kind, was found to be dominant to the Cambodian color. Thus, when a pure Siamese fighting fish of green color is mated to a pure Cambodian, all the offspring show the green color of the Siamese parent.

[1] Read before the Third Philippine Science Convention, February 27, 1935.

If the hybrid Siamese from the above mating are bred together, the resulting offspring are of two kinds in the proportion of three green Siamese to one Cambodian. However, when the hybrid Siamese is mated to a pure Cambodian, the results are of two kinds; namely, green Siamese and Cambodian color in about equal number. Mating pure Cambodian to pure Cambodian has been found to produce nothing but pure Cambodian. The same was found to be true when pure Siamese fishes were mated. Knowing these principles, a breeder can more or less produce the color desired.

Besides color, the vigor of the parents should be considered. Vigorous parents tend to produce strong offspring in which the percentage of mortality is small.

Once the stock is selected, the male must be well fed before mating as it will hardly take any food while caring for the eggs and the young. Likewise, the female should also be fed well. The selected pair must be housed in separate jars, or aquaria, and placed side by side in such a way that they can see each other. It is preferable that the male be placed in a more or less permanent balanced aquarium. When both are ready to mate, the male will be seen to build a froth nest, which is composed of a thick foam of bubbles. The nest of bubbles when completed, appears as a thick white foam and is slimy or adhesive. While building the nest the male, from time to time, swims in the direction of the female, displaying his brilliant colors. The female, when ready, may be seen responding by showing alternate dark and light bands across her body. Her belly is well distended with the eggs partly visible from the outside. The visibility of the eggs, which are partly protruding from the anal opening, indicates that the female may be placed with the male.

On being placed together, the male will start chasing the female; when the latter is cornered, the former will show his color to the full, with his tail, fins, and operculum fully spread. The female, even if ready, usually requires "taming" or "courting." This process may be continued from six to twelve hours before they finally mate. During the taming process the male reënforces his nest by blowing bubbles at more or less regular intervals until it forms a thick foam. The female usually suffers much from physical injuries during taming or courting. The male attacks her from time to time and often mutilates her scales, tail, and fins. There are several cases

known to the writer where the female died from the wounds inflicted by the male. When the female is not ready to breed, she cannot be tamed at all. She should, therefore, be separated immediately from the male, otherwise she will be killed or become disabled.

Mating.—After the female is won, the two display their colors to the fullest extent with their fins and tails widely extended. Soon they begin to swim closely side by side with their fins unexpanded. The male then bends his body on one side and approaches the female, which does the same thing as a sign of positive response. The male then grasps the female by bending his body over the latter with his operculum touching one side of the female's abdomen while the rest of his body presses the other side. The female reacts by turning around with her ventral side up. The male follows the movement of the female and then tightens his grasp with his head and tail. As the male releases his hold, the female drops motionless for a while and then regains her balance. The male immediately darts for the eggs, which are falling from the vent of the female, and picks them up, one by one, in his mouth. The eggs are then placed in the nest of bubbles by blowing them out under it. Here they appear in a mass the size of which depends upon the number of eggs laid. Sometimes the female helps in the gathering of the eggs. This process of pairing and egg-collecting is repeated over and over until all the ripe eggs are squeezed out of the female. The duration of the mating varies from two to three hours. According to my observations the average duration of egg-laying is about two and one-half hours. The average number of pairings, which take place at an interval of less than a minute, is one hundred per mating period. During the early part of the pairing practically no eggs are laid. From the tenth up to the fortieth pairing, the female lays at the rate of three eggs per pairing. After this, the rate reaches an average of ten eggs per pairing. This rate continues up to almost the end of mating, although in the last few pairings the number of eggs decreases. The average number of eggs laid is five hundred, although this depends somewhat upon the size of the female. Large females produce more eggs than do small ones. While the male has his body coiled around the female, the spermatozoa are being discharged into the water in the neighborhood of the female's vent. Thus, the eggs become fertilized as fast as they are discharged by the female.

Soon after all the mature eggs are laid, the male drives the female away and guards the nest. When this happens, the female must be removed, especially if the breeding aquarium is small. In a large breeding aquarium, with plenty of aquatic plants or rocks where the female can hide, she need not be removed.

I had a case in which two individuals were mated before the nest was ready. When the eggs were laid, the male after picking them up in his mouth, just blew them up with air bubbles in the water. Because of the absence of the froth nest, the eggs were scattered all over the surface of the water. However, when the pairing was over, the male began to construct the froth nest and later gathered the eggs together under the nest.

Care of the eggs.—One of the most interesting features about this fish is that the male cares for the eggs and the young. By continuously blowing bubbles under the foam, he aërates the eggs and at the same time reënforces the nest. He picks up any eggs that fall to the bottom of the aquarium and deposits them in the nest. It is probable that the continuous movement of the male at this time, together with the constant motion of his fins under the foam, helps to aërate the eggs. It is apparent that aëration and care by the male is very essential in the hatching of the eggs. Experience shows that an early separation of the male is detrimental to the development of the eggs. Eggs that are isolated from the batch in the nest fail to develop, presumably because of lack of proper aëration.

During the period of caring for the eggs, the male does not feed at all. He does not eat even if he is given plenty of mosquito larvæ or other kinds of food. He may pick up one or two larvæ, but he does not readily swallow them as he does when not caring for the eggs and the young. This is perhaps an adaptation to prevent him from feeding upon his young. It is, therefore, necessary that he be well fed before mating. When starved the male may devour all the eggs.

The time required for hatching apparently depends on room temperature. The incubation period is shorter when the room temperature is high and longer when it is low. During the hot season it takes about thirty hours for the eggs to hatch.

Care of the young.—The male also exercises great care over the young fry after hatching. The newly emerged fishes are usually attached vertically to the adhesive foam. A slight jarring or movement of the water causes some of them to drop with

their head down. The stronger ones may again reach the top without the help of the male by darting upward in a spiral way. The weaker ones fall directly to the bottom and die if not attended to by the male. It is at this particular period that the presence of the male is absolutely necessary. He darts after them and picks them up, one by one, and later puts them back on the surface. The time required from the hatching of the eggs to the time when the fry can swim horizontally is from thirty-six to sixty hours. It is not advisable to remove the male even a few hours prior to the time when the fry can swim freely. The male may stay with his young indefinitely provided that his association with them is not interrupted. When the male is removed from them for sometime and then is returned, he begins to eat them.

Period of growth.—The newly hatched fry measures about 3 millimeters in length. It does not eat at this time and is nourished by the yolk, which is very conspicuous at the posterior end of its abdomen. Three days later the fry has to be supplied with food as the yolk is entirely absorbed at this time. As it starts to swim actively, it begins to look for food, which consists mostly of *Paramecium* and young *Daphnia.* For a month or more the young fry depends on this food for its proper development. In case there are plenty of *Daphnia,* or water fleas, a large number of them must be placed in the breeding aquarium soon after the fry can swim freely, otherwise the male will mistake his young for food. In a few days, the water fleas will produce young in great quantities, which will serve as food for the fry. *Paramecium* need not be supplied if the water fleas are given. As the fry grow bigger, they feed on larger *Daphnia* and very young mosquito larvæ. The first month is the most delicate period in the life of these fishes. However, so long as they are properly fed with *Paramecium* and other protozoa or with tiny young *Daphnia,* and as long as the water is not too acid, the mortality is small.

After two months sex differences become visible. In the male the ventral fin grows longer and the color is more intense. The fighting instinct may also start to develop at this time. Young of the same age do not usually grow at the same rate and do not mature at the same time. Those that grow fast mature early. It is size rather than age that determines maturity. Some mature as early as three months, while others do not mature even after a longer period of time. Exceptional cases were reported

by Mr. Thorson, of Manila, who was able to raise fighting fishes to maturity in a record time of fifty-six days. The difference in the rate of growth and maturity is mainly due to food. Live and fresh food hasten growth rate and maturity more than does preserved food. A year-old fish which was kept in a small aquarium without proper feeding had the size of one that was only about a month old but properly fed.

Rearing.—An aquarium ranging from 6 by 8 by 12 inches to 12 by 12 by 24 inches serves as a good breeding container. A permanent breeding aquarium must be provided at the bottom with a layer of soil 0.5 to 1 inch thick, followed by a thin layer of gravel and a similar layer of sand. Water should be added in such a way that the sand is not too stirred. This may be done by placing a piece of glass over the layer of sand while the water is being gradually poured. Aquatic plants, such as *Sagittaria, Vallisneria, Elodea, Hydrilla,* and *Cabomba,* may be planted so as to make it a balanced aquarium. In a few days after the water has cleared up, the aquarium may be ready for breeding purposes. In this type of aquarium the water need not be changed.

If the breeding aquarium is small, some of the fry may be transferred to other aquaria after one or two weeks. However, if the aquarium is large, the fry need not be transferred to another container. Here they can be kept for a month or two when properly fed. Afterwards, when they are about one or more centimeters long, they may either be transferred en masse to a larger tank or be placed singly in good-sized wide-mouth bottles. With proper care and feeding the rate of growth of fishes in these bottles is remarkable. It is with this isolation method that Mr. Thorson was able to induce maturity in a much shorter time than is usual. When the mass method is preferred to the isolation method, they may be transferred to a good-sized glass or concrete tank where they may be left together indefinitely. It is not advisable to place together young of different sizes belonging to different batches. When they differ greatly in age and size, the older and larger ones eat or harm the smaller ones. Moreover, during feeding the large ones have a decided advantage over the small ones.

Both the isolation and mass method of rearing the young after they are transferred from the breeding aquarium are effective and successful. The only difference between them is that in the isolation method more care and attention are needed. In mass

culture one can leave them in the tank with very little care and attention.

Food.—The most important problem to be encountered in the rearing of the young is food. The rate of growth of the young depends on the amount and kind of food they get. From the time the fry is able to swim freely and horizontally it begins to look for food. At this stage it should be given enough *Paramecium.* A culture of *Paramecium,* therefore, must be ready before the eggs are hatched. It can be prepared in many ways. The most effective method is by placing dried grass or hay in glass or battery jar containing pond water. The jar is then covered with a glass plate to prevent mosquitoes from laying eggs in it. Other materials like dried banana, papaya leaves, fresh grass, and lettuce leaves may be substituted for hay. Hay extract also can be used, provided some paramecia are introduced later after it has cooled. Water plants and algæ, such as *Hydrilla, Elodea, Chara, Spirogyra,* etc., when placed in a glass bowl in large quantities, will also serve as a good medium for the growth of *Paramecium.* It is easy to detect whether the culture is rich or poor by placing a sample of the water from the culture in a clear glass bottle and then examining it against the light. The appearance of a white cloudy scum on the surface and along the wall of the container usually indicates the presence of a large number of Protozoa. A good substitute for paramecia are the young of *Daphnia* which have been mentioned previously.

In feeding the fry with these paramecia utmost care must be exercised. A clean pipette must be used to remove them with the least amount of the liquid medium, otherwise, the water in the aquarium will be very acid. The success in obtaining the greatest percentage of survivors during this critical stage in the life of the fry depends on the technic of feeding. They must be fed with paramecia at least twice a day for about a month. After this period the food may be changed to newly hatched mosquitoes or young *Daphnia.* The food which gives the best result are the young *Daphnia.* These little crustaceans occur in nearly all bodies of fresh water. The success of the ornamental fish breeder depends upon these little crustaceans as they are the best food for all ornamental fishes in any stage of their development, from fry to adult.

Therefore, one must have a constant supply of these crustaceans in order to assure his success. These food materials may

be collected from time to time in open water reservoirs, ditches, ponds, and rice paddies. These sources, however, are not very dependable because they usually dry up during the hot season. The enterprising breeder must not, therefore, depend upon the natural source of these crustaceans, but must resort to artificial cultivation in order to maintain their constant supply.

Recently my attention has been attracted by the success of Mr. Manalang of the University of the Philippines in obtaining almost complete success by feeding the fry only with *Daphnia* and not with infusoria. This was done by placing a large number of *Daphnia* with the fry soon after the male was removed. *Daphnia* lay eggs from which emerge young which serve as excellent food for the fry. I repeated the same technic and I succeeded in obtaining a large number of young which reached their medium size in a little over a month. Although this technic is successful with the fighting fishes, as well as with paradise and dwarf gourami, it is not altogether a success with other fishes, like the zebra.

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